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(54) Title: LAMININ 15 AND USES THEREOF

(57) Abstract: The present invention features a novel member of the laminin family, i.e., laminin 15, the methods of making these molecules, and the methods of using these molecules in treating neural disorders, e.g., retinal disorders.

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LAMININ 15 AND USES THEREOF

Background of the Invention

Laminins are large heterotrimeric glycoproteins of the extracellular matrix. Each laminin heterotrimer is composed of an α, a β, and a γ chain, chosen from a number of possible homologues of each chain. Currently, eleven laminin chains have been identified: five α chains, three β chains, and three γ chains (Timpl (1996) *Curr Opin Cell Biol* 8: 618-624).

Summary of the Invention

The invention is based, in part, on the discovery of a novel member of the laminin family, laminin 15. Accordingly, the invention features a purified or isolated preparation, a recombinant preparation, or a composition of laminin 15, which includes laminin chains α5, β2, and γ3. In a preferred embodiment, the laminin 15 is a trimer of an α5, β2, and γ3 chain. In a preferred embodiment the laminin 15 is human laminin 15.

In a preferred embodiment the α5 chain has a molecular weight of 380 kD, or 330 kD, the β2 chain has a molecular weight of 190 kD or 170 kD, the γ3 chain has a molecular weight of 220 kD, 200 kD or 170 kD.

In another preferred embodiment, the α5 chain is reactive with or specifically binds an α5-specific antibody, e.g., the mouse monoclonal antibody 4C7 (Engvall et al. (1986) *J Cell Biol* 103:2457-2465), or an antibody of the same laminin chain-specificity, e.g., one which can compete for the 4C7 epitope. In another preferred embodiment, the β2 chain is reactive with or specifically binds a β2 specific antibody, e.g., a guinea pig polyclonal GP1 (Sanes et al. (1990) *J Cell Biol*111:1685-1699), mouse monoclonal C4 (Sanes et al. (1983) *Cold Spring Harb Symp Quant Biol* 48: 667-678), or an antibody of the same laminin chain-specificity, e.g., one which can compete for the GP1 or C4 epitope. In another preferred embodiment, the γ3 chain is reactive with or specifically binds γ3 specific a antibody, e.g., the rabbit antibody R16 or the rabbit antibody R21 (Koch et al. (1999) *J Cell Biol* 145: 605-618), or an antibody of the same laminin chain specificity, e.g., one which competes for the R16 or R21 binding site.

In yet another preferred embodiment, the α5 chain has the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4. In a preferred embodiment, the α5 chain is at least 60%,

65%, 70%, 75%, 80%, 85%, 90%, 95%98%, 99% homologous to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4. In a preferred embodiment, the α5 chain differs from the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, by at least one, but less than 5, 10, 15 amino acid residues, e.g., by at least one, but less than 5, 10, 15 non-essential amino acid residues. Preferably, the α5 chain retains the ability to form a heterotrimer with the β2 chain and the γ3 chain.

In another preferred embodiment, the β2 chain has the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 8. In a preferred embodiment, the β2 chain is at least 60%, 65%, 70%m, 75%, 80%, 85%, 90%, 95%, 98%, 99% homologous to the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 8. In a preferred embodiment, the β2 chain differs from the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 8, by at least one, but less than 5, 10, 15 amino acid residues, e.g., by at least one, but less than 5, 10, 15 non-essential amino acid residues. Preferably, the β2 chain retains the ability to form a heterotrimer with the α5 chain and the γ3 chain.

In another preferred embodiment, the γ3 chain has the amino acid sequence of SEQ ID NO: 10. In a preferred embodiment, the γ3 chain is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%99% homologous to the amino acid sequence of SEQ ID NO: 10. In a preferred embodiment, the γ3 chain differs from the amino acid sequence of SEQ ID NO: 10, by at least one, but less than 5, 10, 15 amino acid residues, e.g., by at least one, but less than 5, 10, 15 non-essential amino acid residues. Preferably, the γ3 chain retains the ability to form a heterotrimer with the α5 chain and the β2 chain.

In another aspect, the invention features, a purified or isolated preparation, a recombinant preparation, or composition of laminin 15, which includes laminin chains α5, β2, γ3. In a preferred embodiment, the laminin 15 is a trimer of an α5, β2, and γ3 chain. In a preferred embodiment, the laminin 15 is human laminin 15.

The laminin chains of any laminin as disclosed herein can be the initial translation product or a degradation product, e.g., a naturally occurring degradation product of a laminin chain.

In another aspect, the invention features an isolated nucleic acid, e.g., DNA, RNA or cDNA encoding laminin 15, i.e., which encodes α5, β2, or γ3. The isolated nucleic acid can be a combination of nucleic acids each encoding one or more laminin 15 chains or a single nucleic acid, e.g., if in a vector, one or more of the chains can be in one vector or each chain

can be in a separate vector. The α5 can be, e.g., any α5 chain described herein. In a preferred embodiment, the nucleic acid encoding the α5 chain has the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3. In a preferred embodiment, the nucleic acid encoding the α5 chain has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% homology, or has the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3. In another preferred embodiment, the nucleic acid encoding the α5 chain hybridizes, e.g., hybridizes under stringent conditions, to the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3. The β2 chain can be, e.g., any β2 chain described herein. In a preferred embodiment, the nucleic acid encoding the β2 chain has the nucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In a preferred embodiment, the nucleic acid encoding the β2 chain has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% homology, or has the nucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In another preferred embodiment, the nucleic acid encoding the l32 chain hybridizes, e.g., hybridizes under stringent conditions, to the nucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 7. The γ3 chain can be, e.g., any γ3 chain described herein. In a preferred embodiment, the nucleic acid encoding the γ3 chain has the nucleotide sequence of SEQ ID NO: 9. In a preferred embodiment, the nucleic acid encoding the γ3 chain has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% homology, or has the nucleotide sequence of SEQ ID NO: 9. In another preferred embodiment, the nucleic acid encoding the γ3 chain hybridizes, e.g., hybridizes under stringent conditions, to the nucleotide sequence of SEQ ID NO: 9

In a preferred embodiment, the isolated nucleic acid can be expressed in one or more vectors, e.g., an expression vector or expressed directly in a cell. A vector (or vectors) containing a sequence corresponding to the sequence of the isolated nucleic acid can express the isolated nucleic acid in a suitable cell or a suitable in vitro environment.

In another aspect, the invention features producing laminin 15 from a cell transfected with nucleic acid encoding a laminin 15, e.g., a laminin 15 described herein.

In another aspect, the invention features producing laminin 15 from a cell transfected with nucleic acid which encodes one or more of an α5 chain, a β2 chain and/or a γ3 chain, e.g., a nucleic acid described herein.

In another aspect, the invention features a recombinant laminin 15 which can be produced, e.g., by expressing the laminin chains of laminin 15 in a suitable cell host and under a condition suitable for the laminin chains to form laminin 15.

In a preferred embodiment, the laminin 15 differs from a naturally occurring laminin 15 by at least 1, but less than 5, 10, or 15 amino acid residues. In another embodiment, one, two, or each laminin chain of a laminin, differs from its naturally occurring counterpart by at least 1, but less than 5, 10, or 15 amino acid residues.

The invention provides a method for treating a disorder associated with abnormal functions of synapses, e.g., insufficient stability, viability, formation, and/or defective organization of synapses. The method comprises administering to a subject an effective amount of: laminin 15, laminin 14, or a combination thereof.

The invention further provides a method for modulating retinal development, e.g., in the subretinal space, in the interphotoreceptor matrix, and/or in the outer plexiform layer. The method comprises administering to a subject an effective amount of: laminin 15, laminin 14, or a combination thereof.

The invention provides a method for treating a disorder associated with: insufficient neural cell growth, healing and regeneration, e.g., axon outgrowth; a disorder associated with abnormal subretinal space or interphotoreceptor matrix (IPM) such as inadequate stability of IPM; a disorder associated with retina contact, continuity, and/or adhesion; a disorder associated with abnormal and/or insufficient formation of synapses; a disorder associated with viability of a neural cell, e.g., photoreceptor or an element thereof, e.g., outer segment, inner segment, cell body, and/or synapses. The method comprises administering to a subject an effective amount of laminin 15, laminin 14, or a combination thereof.

Another feature of the present invention provides a method of treating a disorder associated with retinal abnormality, e.g., rod dystrophy, rod-cone dystrophy, macular degeneration, retinitis pigmentosa, or retinal detachment. The method includes administering to a subject an effective amount of: laminin 15, laminin 14, or a combination thereof.

Another feature of the present invention provides a method of inducing neural cell growth and/or regeneration, e.g., axon outgrowth. The method includes administering to a subject an effective amount of laminin 15, laminin 14, or a combination thereof. In a preferred embodiment, the method can be used to induce neural cell growth or regeneration in the central nervous system (CNS) and/or the peripheral nervous system (PNS).

In a preferred embodiment, the method includes administering to a wound an effective amount of: laminin 15, laminin 14, or a combination thereof.

Still another feature of the invention provides a method of promoting a condition,

e.g., promoting retina inter-photoreceptor matrix stability; promoting the production, stability, and/or development of a retina photoreceptor or an element thereof, e.g., outer segment, inner segment, cell body, and/or synapses; promoting retinal contact, continuity, and/or adhesion; promoting the stability of synapses; and/or promoting the formation of synapses. The method includes administering an effective amount of: laminin 15, laminin 14, or a combination thereof.

Another feature of the invention provides a method for preparing an implant. For example, a method of preparing an implantable tip, an implantable catheter, a retinal implant, a timed releasing device, a neural cell growth guide, an artificial tissue, an implant of the central nervous system, or an implant of the peripheral nervous system. The method includes contacting, e.g., coating or incubating, the implant with laminin 15. In a preferred embodiment, laminin 15, laminin 14, or combinations thereof, can be used for treatment of a damaged eye, e.g., to increase photosentivity in an eye, e.g., by implanting a tip coated with laminin 15, laminin 14, or a combination thereof, into the eye.

In a preferred embodiment, the implant is a subretinal implant, e.g., subretinal microphotodiodes, a visual prosthesis, e.g., a photoreceptive prosthesis (e.g., as reviewed in Peachey, *J Rehabil Res Dev* (1999) 36(4):371-6), an implant for photoreceptor replacement, a phototransistor, or a subretinally implanted microphotodiode array (MPDA) implant. Such implants are described in Zrenner et al. (1997) *Ophthalmic Res* 29(5):269-80; Zrenner et al. (1999) *Vision Res* 39(15):2555-67, or in the abstract entitled "Can Subretinal Microphotodiodes Successfully Replace Degenerated hotoreceptors?" submitted by E. Zrenner et al. at the Vision Research Conference held on May 9, 1998. An example of a corneal keratoprosthesis (the Aachen-Keratoprosthesis) is described in Kompa et al. (2000) *Int J Artif Organs* 23(5):345-8.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject laminin 15 is provided. The method includes: contacting the compound with the subject laminin 15; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject laminin 15. This method can be performed in vitro, e.g., in a cell free system, or *in vivo*. This method can be used to identify naturally occurring molecules which interact with subject laminin 15. It can also be used to find natural or synthetic inhibitors of subject laminin 15. Screening methods are discussed in more detail below.

In one embodiment, an assay is a cell-based assay in which a cell which expresses laminin 15 or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate laminin 15 activity is determined.

The ability of the test compound to modulate laminin 15 binding to a compound, e.g., a laminin 15 substrate, or to bind to laminin 15 can also be evaluated.

Soluble and/or membrane-bound forms of isolated proteins (e.g., laminin 15 or biologically active portions thereof can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$,, 3-[(3-cholamidopropyl)dimethylamminio]-l-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl) dimethylamminio]-2-hydroxy-l-propane sulfonate (CHAPSO), or N-dodecyl=N, N-dimethyl-3-ammonio-1 -propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

In a preferred embodiment, the assay includes contacting laminin 15 or biologically active portion thereof with a known compound which binds laminin 15 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with laminin 15, wherein determining the ability of the test compound to interact with laminin 15 includes determining the ability of the test compound to preferentially bind to laminin 15 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

In another aspect, the invention provides, a method of determining if a subject is at risk for a disorder, e.g., a disorder described herein.

In a preferred embodiment, the disorder is related to a lesion in or the misexpression of a gene which encodes one or more of a laminin 15 chain, e.g., one or more of an α5 chain, the β2 chain, and/or γ3 chain.

Such disorders include, e.g., a disorder associated with the misexpression of a laminin 15 chain, a disorder associated with the central nervous system and/or the peripheral nervous system, a retinal disorder.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene;

detecting, in a tissue of the subject, the misexpression of one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of laminin 15.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO: or naturally occurring mutants thereof or 5'or 3' flanking sequences naturally associated with one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of laminin 15.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of one or more of a laminin 15 chain gene, e.g., one or more of an α5 chain, the β2 chain, or γ3 chain gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to one or more of the α5 chain, the β2 chain, or γ3 chain, or a nucleic acid, which hybridizes specifically with the gene.

In another aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted expression of one or more of a laminin 15 chain or a laminin 15 activity, by administering to the subject laminin 15 or an agent which modulates expression of one or more laminin 15 chain or at least one laminin 15 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted laminin 15 activity or expression of one or more laminin 15 chain can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the laminin 15 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of laminin 15 aberrance, for example, a laminin 15 agonist or laminin 15 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some laminin 15 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in 'the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

The term "effective amount" means the amount that is sufficient to reduce or alleviate at least one adverse effect or symptom of a disorder and/or to induce or enhance at least one biological activity of laminin 15. A biological activity of laminin 15 includes one or more of the ability to: 1) modulate retinal development, e.g., in the subretinal space, the interphotoreceptor matrix, the outer plexiform layer; 2) modulate, e.g., promote, neural cell growth and regeneration, e.g., axonal outgrowth; 3) modulate, e.g., promote, adhesion between cells and/or extracellular matrix, e.g., retinal contact; 4) modulate, e.g., promote,

synaptic formation; 5) modulate, e.g., promote, viability of a neural cell, e.g., a neural retinal cell, e.g., a photoreceptor or an element thereof, e.g., outer segment, inner segment, cell body or synapses; 6) interact, e.g., form a complex, with a dystrophin and/or a P-dystroglycan. An effective amount can be determined by one skilled in the art, e.g., based on the disease stage, age, sex, and weight of the to be treated subject and the condition of the treatment. As a reference, the amount administered can be at a concentration of at least from about 0.1 to 500 pg/ml, from about 1 to 200 g/ml, from about 10 to 150 g/ml, or from about 10 to 100 g/ml.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3'ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley &Sons, N. Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1%SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent

hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1%SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO: 1,3, 5, 7, or 9, or corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of laminin 15 having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-laminin 15 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-laminin 15 chemicals. When the protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1 .0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of a laminin 15 chain (e.g., the sequence of SEQ ID NO: 1, 3, 5, 7, or 9) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present which mediate assembly and are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is

replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), betabranched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine) . Thus, a predicted nonessential amino acid residue in a protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a laminin 15 chain coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for a laminin 15 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO: 1, 3, 5, 7, or 9, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least and even 60%, more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J Mol Biol* (48): 444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8,6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna. CMP matrix and a gap weight of 40, 50,60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4: 1 1-1 7 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at nonwild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

A "purified preparation of cells", as used herein, refers to, in the case of plant or

animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

The term "subject" as used herein refers to a mammal. Examples of mammals include human and nonhuman primates, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, having a disorder associated with insufficient laminin, e.g., laminin 15 activity. The mammal is preferably a primate, e.g., a human.

As used herein the term "administering" refers to delivery of a preparation, composition, an active portion, or an active fragment of laminin 15 alone, in combination with another laminin (e.g., laminin 5, laminin 14) and/or at least one other compound or preparation.

The term "stability" means structural, anatomic molecular, and/or functional integrity, intactness, or completeness which is testable or observable by any suitable means. For example, the stability of retina photoreceptor can be tested by ERG, e.g., indicated by a wave and b wave.

The term "pharmaceutically acceptable carrier" is intended to include a solvent, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used in the composition, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, proprionates, malonates, or benzoates. The composition can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents.

Liposomes, such as those described in U.S. 5,422,120; WO 95/13796; WO 91114445; or EP 524,968 Bl, can also be used as a carrier. Typically, the therapeutic laminin composition is prepared as an injectable, either as a liquid solution or suspension; however, solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an entericcoated tablet or gel

capsule according to known methods in the art, such as those described in U. S. 4,853,230; EP 225,189; AU 9,224,296; and AU 9,230,801.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Brief Description of the Figures

Fig. 1A-1F depicts the nucleotide sequence of murine α5 (SEQ ID NO: 1) and the amino acid sequence of murine α5 (SEQ ID NO: 2).

Fig. 2A-2B depicts the nucleotide sequence of human α5 (SEQ ID NO: 3) and the amino acid sequence of human α5 (SEQ ID NO: 4).

Fig. 3A-3D depicts the nucleotide sequence of murine β2 (SEQ ID NO: 5) and the amino acid sequence of murine β2 (SEQ ID NO: 6).

Fig. 4A-4C depicts the nucleotide sequence of human β2 (SEQ ID NO: 7) and the amino acid sequence of human β2 (SEQ ID NO: 8).

Fig. 5A-5B depicts the nucleotide sequence of murine γ3 (SEQ ID NO: 9) and the amino acid sequence of murine γ3 (SEQ ID NO: 10).

Detailed Description

The invention features a novel member of the laminin family, i.e., laminin 15, and methods of making and using this novel laminin, e.g., in neural associated disorders.

In the methods of treating a disorder, such as a disorder described herein, laminin 15 can be administered alone, or in combination with at least one other laminin (e.g., laminin 5 and/or laminin 14) and/or with at least one other compound or preparation. Administration can include local or systemic administration, including injection, oral administration, particle gun, or catheterized administration, and topical administration. Various methods can be used to administer a therapeutic composition of laminin 15 alone, or in combination with one

another laminin, and/or compound, directly to a specific site in the body. For example, a small neural wound can be located and the therapeutic composition can be applied, e.g., once or several times in one or several different locations, within the wound. A therapeutic laminin 15 composition can be directly administered to the surface of a neural wound, for example, by topical application of the composition, or can be injected into the site of a neural wound, e.g., as part of a liquid solution or suspension. X-ray imaging can be used to assist in delivery of laminin 15 to a site, e.g., the site of a neural wound. Combination therapeutic agents, including a laminin 15 protein, a laminin 15 polypeptide, or a subgenomic laminin 15 polynucleotide, and other therapeutic agents, can be administered simultaneously or sequentially. The administration of therapeutic agents can be repeated.

Receptor-mediated targeted delivery of therapeutic compositions containing laminin 15 subgenomic polynucleotides to specific tissues can also be used. Receptor mediated DNA delivery techniques are described in, for example, Findeis et al., 1993, *Trends in Biotechnol. II* 202-0.5; Chiou et al., 1994, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J. A. Wolff, ed.); Wu &Wu., 1988, *J. Biol. Chem.* 263,621-24; Wu et al. (1994) *J Biol Chem* 269, 542-546; Zenke et al. (1990) *Proc Natl Acad Sci U.S.A.* 87:3655-59; Wu et al. (1991) *J Biol Chem* 266:338-42.

Alternatively, a laminin 15 composition can be introduced into human cells ex vivo, and the cells then replaced into the human. Cells can be removed from a variety of locations including, for example, from a selected neural tissue or from an affected organ. Both the dose of the laminin 15 composition and the means of administration can be determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. If the composition contains a laminin 15 protein or polypeptide, effective dosages of the composition are in the range of about 5 pg to about 50 pg/kg of patient body weight, about 50 pg to about 5 mg/kg, about 100 pg to about 500 pg/kg of patient body weight, and about 200 to about 250 pg/kg.

Therapeutic compositions containing a laminin 15 subgenomic polynucleotide can be administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 pg to about 2 mg, about 5 pg to about 500 pg, and about 20 pg to about 100 pg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression should be considered in determining the dosage of laminin 15

polynucleotide required. Where greater expression is desired over a larger area of tissue, larger amounts of laminin 15 subgenomic polynucleotides and/or the same amounts of laminin 15 subgenomic polynucleotides can be readministered, e.g., in a successive protocol of administrations, or several administrations to different adjacent or in close proximity to the targeted tissue portions.

For example, a tumor site may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

Isolation or recombinant production of laminin 15 ($\alpha5\ \beta2\gamma3$)

Laminin 15 consists of an $\alpha5$ chain, a $\beta2$ chain and a $\gamma3$ chain. The laminin chain can be isolated and purified from a natural source, e.g., from a retinal tissue such as the retina inter-photoreceptor matrix, the retina outer plexiform layer, the neural retina, a Müller cell, and/or a preparation of retinal neurons.

Alternatively, laminin 15 can be produced recombinantly or chemically synthesized by conventional methods. The nucleotide and amino acid sequences of the laminin chains are known and described, for example, at Genbank Accession Number U37501 (murine $\alpha5$ chain), Genbank Accession Number: AW4 11963 (murine $\beta2$ chain), and in Koch et al., J *Cell Biol* (1999) 145: 605-618 (murine $\gamma3$ chain).

Methods of generating a recombinant laminin 15 protein are well known in the art. For example, the laminin 15 protein can be generated by cloning the nucleic acid sequence encoding each of the laminin chains into an expression vector, where it is operably linked to one or more expression control sequences.

A vector can include one or more of an $\alpha5$ chain, the $\beta2$ chain, and $\gamma3$ chain nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the

invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., laminin 15 proteins, mutant forms of laminin 15 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of laminin-15 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

The laminin 15 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1 (1) 1986.

In another aspect, the invention features, a cell or purified preparation of cells which include one or more exogenously introduced laminin 15 chain nucleic acid, e.g., one or more of an $\alpha 5$ chain, the $\beta 2$ chain, and/or $\gamma 3$ chain, or which otherwise misexpress one or more laminin 15 chain. The cell preparation can consist of human or non-human cells, e.g., rodent

cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells includes one or more of a laminin 15 chain nucleic acid, e.g., a heterologous form of a laminin 15 chain nucleic acid, e.g., a gene derived from humans (in the case of a non-human cell). The laminin 15 chain or chains can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include one or more gene(s) which misexpress an endogenous laminin 15 chain, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or misexpressed laminin 15 chain alleles or for use in drug screening.

In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject laminin 15 chain polypeptide.

Also provided are cells, preferably human cells, e.g., human hematopoietic or fibroblast cells, in which one or more endogenous laminin 15 chain is under the control of a regulatory sequence that does not normally control the expression of the endogenous laminin 15 chain gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous laminin 15 chain gene. For example, an endogenous laminin 15 gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published in May 16, 199 1.

Implants

Implants described herein can be neural implants, e.g., neuromuscular stimulators; auditory prostheses, e.g., speech processors; or retinal implants. Preferred neural implants are retinal implants. Retinal implants can be, e.g., subretinal or epiretinal. Subretinal devices, e.g., MPDAs, are less than 1 cm, e.g., approximately 2 millimeters, in diameter and can be composed of tiny electrodes that are powered by a large number, e.g., 3,500, microscopic solar cells. Subretinal devices include the Optobionics™ silicon chip, in which light coming into the eye both powers the device and is transmitted to the brain as an image

by the device. Epiretinal devices can go on top of a damaged retina. A retinal implant can also be a biocompatible device or material designed to carry or deliver a compound or composition to the retina, e.g., an implantable tip, catherer, or tissue; or an electronic device that replaces photoreceptor function, e.g., a phototransistor.

Such implants can be contacted, e.g., coated, with the compositions described herein, e.g., for use in a subject.

Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of laminin 15 and for identifying and/or evaluating modulators of a laminin15 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which one or more of an endogenous laminin 15 chain gene, e.g., one or more of a $\alpha 5$ chain, the $\beta 2$ chain, or $\gamma 3$ chain, has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Examples

Example 1: Laminin Expression

Antibodies which recognize the eleven known laminin chains were used to catalog the laminin chains in adult rat and human retina. The reactivity for antibodies directed against each of these chains was assessed using immunohistochemistry (Libby et al., *Invest Ophthalmol Vis Sci* (1996) 37: 165 l-1661; Libby et al., *J Comp Neural* (1997) 389: 355-367). Adult rat eyecups were embedded in O.C.T. compound (Miles, Elkhart, IN) and frozen by immersion in liquid nitrogen-cooled isopentane. Transverse, 10 pm thick sections, were

cut with a Leica cryostat and placed onto Superfrost Plus slides (Fisher, Pittsburgh, PA). Human retina specimens were obtained as unfixed transverse sections. Slides were stored at --20°C until use. For use, slides were returned to room temperature, immersed briefly in acetone (or, interchangeably, for all but the α5,93, and γ2 chains, MeOH) at -20 °C, washed in phosphate-buffered saline (PBS; 137 mM NaCl, 2.68 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4), and then incubated in primary antibodies for two hours at room temperature or overnight at 4°C. Primary antibodies were diluted in PBS containing 2% goat serum, or 2% bovine serum albumin, or both. Sections were washed in PBS and incubated in species-appropriate, affinity purified, fluorescently-labeled secondary antibodies diluted in 2% goat serum in PBS for 1 hour at room temperature. Following washes in PBS, slides were mounted in 90% glycerol and 10% water, containing paraphenylenediamine (1 mg/ml; Sigma, St. Louis, MO) to reduce photobleaching or in Prolong (Molecular Probes, Eugene, OR). The antibodies used were: laminin 1, ccl Ply1 (Life Technologies; rabbit polyclonal); laminin cr2 chain (Life Technologies; mouse monoclonal); laminin α3 chain (BM-2, made in one of our laboratories (REB); mouse monoclonal); laminin α4 chain (Miner et al. (1997) *J Cell Biol* 137:685-701; rabbit polyclonal and R17, made in one of our laboratories (REB); rabbit polyclonal), laminin α5 chain (Miner et al. (1995) *J Biol. Chem.* 270: 28523-28526; rabbit polyclonal and 4C7, Engvall et al. (1986) *J Cell Biol* 103: 2457-2465; Tiger et al. (1997) J Biol. Chem. 272: 28590-28595); laminin βl chain (C21, Sanes and Chiu; *Cold Spring Harbor Symp. Quant. Biol.* (1983) 48:667-678; mouse monoclonal); laminin β2 chain (GPl, Sanes et al., *J Cell Biol.* (1990) 111:1685-1699; guinea pig polyclonal, C4, Sanes and Chiu, (1983) *supra*; mouse monoclonal and D5, Hunter et al., *Nature* (1989) 338:229-234; mouse monoclonal); laminin β3 chain (6F12, Rouselle et al. (1991) *J Cell Biol* 114:567-576, mouse monoclonal); laminin γl chain (D18, Sanes et al. (1990) *supra*; mouse monoclonal); laminin γ2 chain (Sugiyama et al. (1995) *Eur. J Biochem.* 228:120-l 28; rabbit polyclonal); laminin γ3 chain (R16, R21, Koch et al. (1999) *J Cell Biql* 145:605-618; rabbit polyclonals); laminin 5, α3β3γ2 (4101, Rouselle et al. (1991) *supra*; Marinkovich et al., *J Biol Chem* (1992) 267:17900-17906, 8Ln5 and 9Ln5, made in one of our laboratories (REB); rabbit polyclonals). 8Ln5 and 9Ln5 were made to the same antigen as the published antiserum 4101 and have the same reactivity.

Laminin Alpha Chains

Results showed that a polyclonal antiserum which recognized the three chains of laminin 1 (αl βlγl) reacted only with the vasculature in the rat and human, and not with the matrix of the neural retina itself. Laminin 1 immunoreactivity was seen on the basal side of the retinal pigmented epithelium, i.e., Bruch's membrane; and in those sections in which the inner limiting membrane was present. Laminin 1 was expressed there as well. These observations suggested that the laminin α1 chain, a component of laminin 1, was not associated with the matrix of either the neural retina or the IPM but was a component of the basement membranes of the retina: Bruch's membrane and the internal limiting membrane.

The laminin α2 chain was also present in the retinal vasculature, but was not detected as being associated with ganglion cell bodies or associated with this basement membrane. The α2 chain does not appear to be a component of Bruch's membrane.

In contrast, the laminin α3 chain was present in the interphotoreceptor matrix, prominent .at the external limiting membrane and at the tips of the photoreceptor inner segments. Laminin α3 chain immunoreactivity was also present in the outer plexiform layer. However, in contrast to the chains of laminin 1 and the laminin α2 chain, which were associated with elements of the vasculature in the outer plexiform layer, the laminin α3 chain did not appear to be associated with the larger vessels in this region. Nevertheless, the laminin α3 chain did appear to be present in the outer plexiform layer. It was difficult to discern whether the laminin α3 chain was associated with small vessels or associated with the synaptic connections in this layer. In the human retina, weak immunoreactivity for the laminin α3 chain was also present surrounding cell bodies of the outer and inner nuclear layers. Finally, in human retina, the laminin α3 chain is diffusely associated with the inner plexiform layer.

In contrast to the laminin αl-3 chains, the laminin α4 chain appeared to have a broad distribution in rat and human retina. Immunoreactivity for the laminin α4 chain was present in the IPM, as well as diffusely in both the inner and outer plexiform layers. This extensive immunoreactivity in both plexiform layers, and the lack of any association with the retinal vasculature, suggested that the laminin α4 chain is contained within the extracellular matrix of the plexiform layers. However, the most prominent reactivity for the laminin α4 chain was in what appeared to be Müller cell fibers coursing through the retina. These fibers were confirmed as Müller cell processes, based on co-localization of the laminin α4 chain with a Müller cell marker (vimentin). Reactivity for the laminin α4 chain was also present in the

ganglion cell layer which may reflect that laminin α4 chain associated with the endfeet of Müller cells. The presence of the laminin α4 chain within the Müller cell suggested that the Müller cell was a source of the laminin α4 chain in the neural retina, consistent with the data that confirmed the Müller cell as a source of another laminin chain, β2 (Libby et al. (1997) *supra*).

Our initial localization studies using a polyclonal antiserum raised against the laminin α5 chain (Miner et al. (1995) *supra*) suggested that the laminin α5 chain was only a component of the true basement membranes of the retina, i.e., the internal limiting membrane, Bruch's membrane, and vascular basement membranes. However, a monoclonal antibody that specifically recognizes the laminin α5 chain (4C7, Engvall et al. (1986) *supra*; Tiger et al. (1997) supra) demonstrated that the laminin α5 chain is more broadly distributed within the neural retina: the laminin α5 chain had a distribution similar to that for the laminin γ3 chain. Specifically, the laminin α5 chain was present in both rat and human interphotoreceptor matrices, as well as in the outer plexiform layer in the rat. In addition, the laminin α5 chain, like the laminin α1 and α2 chains, was associated with the retinal vasculature. This was particularly notable in the human. Laminin α5 chain immunoreactivity was present in the choroid, the hyaloid vessels, the outer plexiform layer vessels and the vasculature which extends through the retina from the hyaloid vessels to the outer plexiform layer. This expression in the vasculature was similar to the expression pattern for the laminin α5 chain in the brain.

Together, these data suggested that all five laminin α chains were expressed in the retina, but two--the laminin α1 and α2 chains--may be associated exclusively with the retinal vasculature. In contrast to these two laminin α chains, three chains, the laminin α3, a4 and α5 chains, were associated with the IPM and, potentially, associated with the neural retina at synapses in the plexiform layers. Laminins at each of these locations could be provided from the cell that spans the entire thickness of the retina, the Müller cell; the Müller cell is the likely source for at least one other laminin chain, β2 (Libby et al. (1997) *J Comp Neural* 389:355-367).

Laminin Beta Chains

As noted above, a polyclonal serum that recognized all three chains of laminin 1, including the laminin β1 chain, reacted only with the vasculature in rat and human retina. For

the human retina, this pattern was consistent with the previously reported expression of laminin 1 (Toti et al. (1997) *Neuromusc Disord* 7:2 1-25). Thus, the laminin β1 chain was not an element of the matrix of either the IPM or the neural retina. A rat reactive antibody against the βl chain confirmed this observation. However, as there was little authentic laminin αl chain in the retina, and little authentic laminin βl chain in the retinal vasculature of the rat, it was likely that the polyclonal serum against laminin 1 was detecting largely the laminin γl chain in the vasculature of both rat and human.

As previously reported in the rat (Libby et al. (1997) *supra*), the laminin β2 chain was present in the interphotoreceptor matrix, and appeared to be associated with the external limiting membrane. Here, a similar distribution in the human retina was demonstrated. The laminin β2 chain, a known component of brain vasculature, was also associated with the vessels of the retina. In the human, immunoreactivity was also present surrounding cell bodies in the inner nuclear layer, as well as in the inner limiting membrane. In both species, the laminin β2 chain was also diffusely associated with the outer plexiform layer. A comparison of this diffuse immunoreactivity to that for laminin 1 or the laminin α2 chain suggested that the laminin β2 chain was not only associated with the vasculature within the outer plexiform layer. The laminin β2 chain may also be associated with the extracellular matrix of the outer plexiform layer and localized to synapses in the central nervous system, as it is in the peripheral nervous system (Hunter et al. (1989) *Nature* 338:229-234).

Laminin β3 chain immunoreactivity was also present in the mature rat retina, as well as the mature human retina. The β3 chain seems largely limited to the inter-photoreceptor matrix, suggesting that laminins containing the laminin β3 chain are components of this matrix. As laminin β3 has a tightly restricted tissue distribution in rodent (Utani et al. (1995) *Lab Invest* 72: 300-310), and has, so far, only been demonstrated as a component of laminin 5 (α3β3γ2), it is likely that this reflects the presence of laminin 5 in the interphotoreceptor matrix.

Together, these data suggested that, although the laminin βl chain was associated with the basement membrane of the retinal vasculature in both rat and human retina, only two β chains, the laminin β2 and β3 chains, were expressed in the matrix of the IPM. Moreover, the laminin β2 chain was also expressed in the matrix of the outer plexiform layer.

Laminin Gamma Chains

As noted above, a polyclonal serum that recognized all three chains of laminin 1, including the laminin γ1 chain, reacted largely with the vasculature. Consistent with this observation, an antibody directed against the laminin γ1 chain reacted only with the vasculature in both rat and human, suggesting that the anti-laminin 1 serum was reacting with at least the γ1 chain. In addition, in the human, the laminin γ1 chain was present at the internal limiting membrane. This may reflect production by astrocytes, the hyaloid blood vessels, and retinal ganglion cells (Sarthy and Fu (1990) *J Cell Biol* 110: 2099-2108; compare Sarthy, *Vis. Sci.* (1993) 34: 145-152). There was also some punctate immunoreactivity for the laminin γ1 chain within the ganglion cell layer. Importantly, there was no laminin γ1 chain reactivity in the IPM or plexiform layers. Thus, the laminin γ1 chain was confined to the vitread side of the retina.

In contrast to the laminin γ1 chain, the laminin γ2 chain was present in the inter-photoreceptor matrix of rat and human retina. It was also present in the hyaloid vessels, and, to a limited extent, the intraretinal capillaries of the human. Some laminin γ2 chain was also present in the outer plexiform layer of the rat; this immunoreactivity may reflect capillary-associated laminins. As for the laminin β3 chain, previous reports have suggested a restricted distribution of the laminin γ2 chain (Kalhmki et al. (1992) *J Cell Biol* 119:679-693).

The laminin γ3 chain was the most recently isolated of the growing family of laminins (Koch et al. (1999) *supra*). The tissue distribution of this chain was quite limited. However, it was most extensively expressed in the nervous system. The results showed the presence of the laminin γ3 chain in a portion of the human and rat central nervous system. Prominent laminin γ3 chain immunoreactivity was present in the interphotoreceptor matrix; notably, throughout the region of photoreceptor inner segments. In addition, there was marked laminin γ3 chain immunoreactivity associated with the external limiting membrane in the rat and surrounding cell bodies within the outer and inner nuclear layers in the human. Finally, the laminin γ3 chain was diffusely present in the outer plexiform layer, at least in the rat. As with the laminin α3, α4, and β2 chains, it cannot be said conclusively that the laminin γ3 chain immunoreactivity in the outer plexiform layer was concentrated at points of synaptic contacts in the outer plexiform layer. However, the laminin γ3 chain was not associated with the vasculature present at the vitread side of the retina, and its pattern of expression was distinct from that. For laminin chains in the vasculature, such as the γ1 chain. Therefore, it

was probable that the laminin γ3 chain in the outer plexiform layer was contained within the matrix of the plexiform layer.

Together, these data suggest that the laminin γ2 and γ3 chains were the only known laminin γ chains in the IPM. Furthermore, the laminin γ3 chain appears to be the only laminin γ chain found potentially associated with the synaptic regions of the outer plexiform layer in both rat and human.

Thus, in the IPM, seven laminin chains: α3, a4, α5, β2, l33, γ2 and γ3 were present. This was consistent with the presence of one previously isolated laminin, laminin 5 (α3β3γ2), as well as several novel laminin heterotrimers. If the other chains were to combine, there would be at least two such novel laminin trimers in the IPM: α4β2γ3 and α5β2γ3. In the matrix of the outer plexiform layer, these two trimers also appear to be present, as their component chains are present. In contrast, only one laminin chain, α4, is prominent in the matrix of the inner plexiform layer, suggesting that other, uncharacterized, β and γ chains may be expressed in the retina.

Example 2: RNA Expression

cRNA probes which recognize the RNAs encoding the eleven known laminin chains were used to catalog these RNAs in the retina and to localize them to particular cell types using *in situ* hybridization. As laminin trimers are assembled prior to secretion, the RNAs encoding all three chains of any given trimer should be present in the same cell.

In situ hybridization was performed as follows. Adult rat eye cups were dissected and fixed overnight at 4°C in 4% paraformaldehyde in PBS (pH 7.4), dehydrated, and embedded in paraffin. Fifteen micron-thick sections were cut and placed onto Probe-on Plus slides (Fisher). Human retina specimens were obtained as fixed transverse sections. Rehydrated rat sections or frozen human sections were then processed for *in situ* hybridizations as previously described (Libby et al. (1997) *supra*).

cRNA probes for the laminin chains were generated as previously described (Libby et al. (1997) *supra*). Probes for the laminin β1 and β2 chains and for cellular retinaldehyde binding protein were those used previously (Libby et al. (1997) *supra*). A cRNA probe for the laminin α5 chain (Miner et al. (1995) *supra*) was generated. cRNAs were labeled during transcription by the incorporation of digoxigenin-UTP (Boehringer Mannheim, Indianapolis, IN); l μg/ml of cRNA was used for hybridization.

Laminin Alpha Chains

RNAs encoding the laminin α1 and α2 chains were not readily detected in the rat or human retina, suggesting that both of these RNAs were not abundant in the retina. However, for both chains, some RNA was detected in the inner nuclear layer. This may reflect production of these two chains by components of the vasculature.

In contrast, the RNA encoding the laminin α3 chain was readily detectable in the rat and human retina. This expression agrees with the high expression levels of the laminin α3 chain in the retina from the human expressed sequence tag database. Interestingly, laminin α3 chain RNA was not localized to perinuclear sites. Rather, the RNA was in fibers coursing through the inner and outer nuclear layers and the outer plexiform layer. This location was consistent with production of laminin α3 chain RNA by Müller cells.

The RNA encoding the laminin cc4 chain was present in a pattern similar to that encoding the laminin α3 chain. The RNA appeared to be located in fibers coursing through the inner and outer nuclear layers, which were likely to be Müller cell processes. Unlike laminin α3 chain RNA, there did seem to be perinuclear laminin α4 chain RNA in the inner nuclear layer, particularly of the human retina, suggesting that the source of the RNA encoding the laminin α4 chain was a cell whose nucleus resides in the inner nuclear layer. Müller cell nuclei were in this layer. Finally, in human retina, laminin α4 chain RNA was present in the ganglion cell layer, in what was presumed to be Müller cell endfeet.

Similar to the laminin αl and α2 chain, RNA encoding the laminin α5 chain was not detectable within the rat retina. This suggests that the RNA encoding the laminin α5 chain was not abundant in the rat retina. In an example of species variation, RNA encoding the laminin α5 chain within the human neural retina of the human was detected. The pattern of expression for laminin α5 chain RNA in the human retina was similar to, albeit considerably less intense than, that detected with a probe for laminin α4 chain RNA.

Together, the patterns of expression for the RNAs encoding the laminin α chains suggest that the laminin α3, α4 and α5 chain RNAs were expressed in the neural retina, consistent with the presence of laminin α3, α4 and α5 chain protein noted above. Specifically, they suggested that laminin α3, α4 and α5 chains were produced in the neural retina and deposited in the matrices of the IPM and outer plexiform layer, and, in the case of the laminin α4 chain, the inner plexiform layer.

Laminin Beta Chains

RNA encoding the laminin β1 chain was not highly expressed in the neural retina, as previously reported (Libby et al. (1997) *supra*). These data were consistent with the lack of laminin β1 chain protein in neural structures within the retina.

It was previously shown that the laminin β2 chain was expressed in the adult rat retina (Libby et al. (1997) *supra*). RNA encoding the laminin β2 chain was present in fibers in the outer and inner nuclear layers of the rat. In the human retina, RNA encoding the laminin β2 chain was present in what appear to be fibers in the inner and outer nuclear layers. It was striking at the external limiting membrane, and was also present in the ganglion cell layer. It had been previously ascribed that the laminin β2 chain RNA was in the ganglion cell layer to Müller cell endfeet (Libby et al. (1997)). There was also perinuclear RNA present in and around some cell bodies in the inner nuclear layer, suggesting a cell in the inner nuclear layer, possibly the Müller cell, was a source of the laminin β2 chain in the neural retina. Finally, as shown here, for the rat, and here for the human, this pattern of RNA expression was similar to that for cellular retinaldehyde binding protein, an authentic marker of the Müller cell (Bunt-Milam and Saari (1983) *J Cell Biol* 97:703-712).

Laminin β3 chain RNA appears to be expressed in the adult rat retina. RNA encoding the laminin β3 chain was located in fibers coursing through the inner and outer nuclear layers, in the outer plexiform layer, and at the outer limiting membrane. In another example of species variation, laminin β3 chain RNA could not be detected within the human neural retina.

Together, these data suggested that, in both rat and human, the laminin β2 chain was the prominent β chain expressed in the neural retina. In addition, the laminin β3 chain appeared to be a component of the neural retina. Finally, the laminin β1 chain was not likely to be expressed in the mature neural retina.

Laminin Gamma Chains

The RNA encoding the laminin γ1 chain could not be detected in the neural retina. This suggested that the laminin γ1 chain protein in the internal limiting membrane was not derived from the neural retina. The laminin γ1 chain in the internal limiting membrane must, therefore, be derived from one of the non-neural retinal cells that contact it. Both astrocytes and the hyaloid vessels contact the internal limiting membrane and have been suggested as

sources for protein components of the internal limiting membrane (Sarthy and Fu (1990) *supra*; Sarthy (1993) *supra*).

RNA encoding the laminin γ2 chain was consistently difficult to detect in the retina. However, the RNA was detectable in the inner nuclear layer of the human retina, and to a lesser extent, the rat retina.

In contrast, RNA encoding the laminin γ3 chain was readily detected in both the rat and human retina. Laminin γ3 chain RNA was expressed in a pattern that is similar to that for several other laminin chain RNAs. In fibers coursing through the outer nuclear layer, at the external limiting membrane, and in presumed Müller cell endfeet in the ganglion cell layer. The γ3 chain was, therefore, a likely y component of mature retinal laminin.

Thus, the expression patterns for the laminin chain RNAs detected in the neural retina demonstrated that RNAs encoding the laminin α3, α4, β2, γ2 and γ3 chains are expressed in the rat and human retina; in addition, RNA encoding the laminin α5 chain was detected in human retina, and that encoding the laminin β3 chain was detected in rat retina. Although slightly different, the basic distribution of all of these RNAs was the same: largely within fibers coursing through the inner and outer nuclear layers. RNAs for the laminin α4 and β2 chains also appear to be present at perinuclear sites in the inner nuclear layer as well as within the ganglion cell layer. Together, these data suggested that the Müller cell is the source of these laminin chain-encoding RNAs. In addition, it supports that the retina produces two novel laminin trimers: laminin 14 (α4β2γ3) and laminin 15 (α5β2γ3).

Example 3: Biochemical Identification of Laminin 14 and 15 in the Retina

The protein and RNA localization data suggested that laminins 5, 14 and 15 were expressed in the neural retina. These findings were extended by isolating laminins, and their component chains, from the retina.

The biochemical isolation of laminin heterotrimers was preformed as follows. Bovine eyes were obtained from Pel-Freez (Rogers, AR) and dissected to isolate the retina. About 50 retina were pooled, washed in PBS containing the protease inhibitors phenylmethylsulfonyl fluoride (150 mg/l) and N-ethylmaleimide (650 mg/l), frozen in liquid nitrogen, ground in a Waring blender and resuspended in 100 ml of 2 M urea, 0.5M NaCl, 10mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM N-ethylmaleimide, and 50mM Tris-HCl (pH 7.8) , then stirred for 24 hours at 4°C. The retinal extract was cleared by

centrifugation at 30,000 g for 60 min, dialyzed in 0.5M NaCl and 5OmM Tris-HCl (PH 7.8), then cleared by centrifugation at 100,000 g for 60 min. Glycoproteins were isolated by applying the extract to a Concanavalin-A-Sepharose column (Pharmacia, Piscataway, NJ). Unbound material was removed by washing with 0.5M NaCl, 5m. M $CaCl_2$, 5 mM $MgCl_2$ and 50mM Tris-HCl (pH 7.4). The column was washed with 10mM α-D-methylmannopyranoside in 0.5 M NaCl and 50 mM Tris-HCl (PH 7.4) and then eluted with 1 M a-D-methylglucopyranoside in 0.5 M NaCl and 50 mM Tris-HCl pH 7.4).

To isolate laminin 14 and laminin 15, the Concanavalin-A eluate was separated without sulfhydryl reduction on a 3-5% polyacrylamide-SDS gel. After staining with Coomassie Brilliant Blue R-250 (Sigma), bands containing the high molecular weight proteins were excised, washed in 0.5 M Tris-HCl pH 6.8), incubated in SDS sample buffer containing 10% P-mercaptoethanol for 30 minutes at ambient temperature, and the different laminin chains were separated on a 5% polyacrylamide-SDS gel. Proteins were analyzed by protein transfer ("Western") blot analysis using an anti-laminin α4 chain antiserum (R17), an anti-laminin β2 chain antibody (D5) , and an anti-laminin γ3 chain antiserum (R21).

The 380 kD protein isolated by this method was not reactive with any of the anti-laminin antibodies. Therefore, following digestion by the protease Lys-C, peptide fragments of this protein were sequenced using matrix-assisted laser desorption time-of-flight mass spectrometry (Chait and Kent (1992) *Science* 257:1885-l 894) performed on a Finnegan Lasermat 2000.

Although the presence of the laminin α3 chain protein on protein transfer blots of retinal extracts was demonstrated, it has been impossible to isolate any heterotrimeric laminins containing the laminin α3 chain from retinal extracts, and have, therefore, been unable to confirm biochemically the presence of laminins 5 (α3β3γ2) or 13 (α3β2γ3). This may reflect a relative dearth of these trimers in the retina, or a difficulty in extracting them in a native form. However, it has been previously shown that the laminin β2 chain is present in retinal extracts (Hunter et al. (1992) *Neuron* 8:399-413). In addition, laminins eluted from an anti-laminin β2 chain resin contain the α4 chain, demonstrating that the β2 chain is associated with at least this chain in the retina.

Retinal laminins were isolated from retinal matrix by chromatography on Concanavalin A-Sepharose followed by size fractionation on polyacrylamide gels. Two high-molecular weight components were selected from this purification scheme. Each was

reduced and separated on polyacrylamide gels. The first (band "A") resolved into components of approximately 190, 220, and 380 kD. Two of these proteins were identified immunologically as the laminin β2 (190 kD) and γ3 (220 kD) chains. The third did not react with any of the anti-laminin antibodies (e.g., anti-α4). The second high molecular weight component (band "B") resolved into components of approximately 190 and 220 kD. The 190 kD component consisted of both the α4 and β2 chains, and the 220 kD component was identified as the γ3 chain. No other chains were detected as components of this complex; therefore, band "B" consists of the novel laminin composed 5 of α4, β2, and γ3 chains, which was term laminin 14.

The high molecular weight of this protein suggested that band "A", was a laminin α chain, perhaps the laminin α5 chain. However, as antibodies did not react with this protein, it was excised from a polyacrylamide gel, digested, and microsequenced. The resultant fragments were compared to known laminin sequences, and all were identical to sequences within the laminin α5 chain (Table One), demonstrating that this third component is the laminin α5 chain. Therefore, band "B" consists of the novel laminin composed of α5, β2, and γ3 chains, which was term laminin 15.

Table One

peptide 1	AHPVSNAIDGTER
mouse laminin α5 (36-48)	AHPVSNAIDGTER
peptide 2	WWQSPPLSR
mouse laminin α5 (53-61)	WWQSPPLSR
peptide 3	FANSPRPDLWVLER
mouse laminin α5 (83-96)	FANSPRPDLWVLER
peptide 4	TNTLLGHLMGK
mouse laminin α5 (188-198)	TNTLLGHLMGK
peptide 5	FGFNPLEFENFSWR
mouse laminin α5 (797-810)	FGFNPLEFENFSWR
peptide 6	LELEEAATPEGHAVR
mouse laminin α5 (813-827)	LELEEAATPEGHAVR
peptide 7	AGALLPAIR
mouse laminin α5 (2099-2107)	AGALLPAIR

peptide 8	KLIAQAR
mouse laminin α5 (2640-2646)	KLIAQAR

Expression of Laminins 5, 14, and 15 During Retinal Development

It was previously shown that the laminin β2 chain was expressed throughout retinal development (Libby et al. (1996) *supra*; Libby et al. (1997) *supra*), at first in the subretinal space, and subsequently in the interphotoreceptor matrix and the outer plexiform layer. In addition, it was recently shown that the laminin β2 chain is critical for proper formation and function of synapses in the outer plexiform layer (Libby et al. (1999) *J Neurosci.* 19: 9399-9411). These observations were extended by examining the expression of potential partners for the laminin β2 chain, i.e., components of laminins 14 and 15, during development of the interphotoreceptor matrix and outer plexiform layer. In the course of these experiments, it was also found that the components of laminin 5 (α3 β3γ2) were expressed in the developing retina. The expression of the components of laminins 14 and 15 were examined, as well as those of laminin 5 (the laminin α3, α4, α5, β2, β3, γ2, and γ3 chains) from postnatal day 0 (P0) through postnatal day 15 (P15) . The appearance of these chains was compared with that for two components of the photoreceptor synapse, dystrophins and pdystroglycan.

At P0, few rod photoreceptors have differentiated in the rat retina, and the outer plexiform layer had not yet formed (and, therefore, no dystrophins are present). At this age, the laminin β2 chain is prominently expressed in the subretinal space, as previously reported at E2 1 (Libby et al., 1996, *supra*), and in fibers spanning the width of the retina, as are the other components of laminins 14 and 15, the laminin α4, α5, and γ3 chains. In addition, the laminin α3, β3, and γ2 chains are also expressed in these locations, and an antiserum against laminin 5 displays similar immunoreactivity. These immunohistochemical data are consistent with the expression of laminins 14 (α4β2γ3) , 15 (α5β2γ3) , and 5 (α3β3γ2) at P0. However, none of the laminin chains were concentrated in the region that will eventually become the outer plexiform layer.

At P5, the central portion of the retina had begun to elaborate an outer plexiform layer, in which dystrophins were expressed, whereas the peripheral portion had not. At this age, the components of laminins 14 and 15 were still present in the subretinal space and in fibers spanning the thickness of the retina. In addition, in the central portion of the retina, these laminin chains were beginning to be concentrated in the developing outer plexiform

layer. Components of laminin 5 remained associated with the subretinal space and in fibers spanning the thickness of the retina. At P10, the entire retina had developed an outer plexiform layer, in which dystrophins were prominently expressed. Interestingly, another component of the adult photoreceptor synapse, β-dystroglycan, was not detectable at this age. The components of laminins 14 and 15 (the α4, α5, β2, and γ3 chains) were concentrated in the developing interphotoreceptor matrix and the outer plexiform layer. In addition, laminin 5 immunoreactivity remained associated with the subretinal space and the outer plexiform layer. In addition, monoclonal antibodies against all three chains of laminin 5 (α3, β3 and γ2) were reactive, suggesting that laminin 5 expression continues.

At P15, the outer plexiform layer was beginning to reach maturity, as judged by the continued presence of dystrophins and now detectable levels of β-dystroglycan. In other respects, the retina at P15 is similar to the adult: the components of laminins 14 and 15, including the laminin α4, β2, and γ3 chains are prominently expressed in the interphotoreceptor matrix and outer plexiform layer, the α4 chain is prominent in fibers spanning the retina, and laminin 5 remains. Expression of one component of the outer plexiform layer, β-dystroglycan, appeared to lag behind the others. By β26, however, the expression closely mimics that of the adult.

In summary, the developing retina contained components of laminins 14 and 15 throughout the period of inter-photoreceptor matrix and outer plexiform layer formation. Initially, these chains were expressed in the subretinal space and in fibers spanning the thickness of the retina. With time, they became more restricted to, the interphotoreceptor matrix and outer plexiform layer, reflecting the distribution present in the adult. In addition, components of laminin 5 are expressed in the interphotoreceptor matrix and outer plexiform layer of the developing retina, but become somewhat restricted to the interphotoreceptor matrix by the time a mature morphology is attained. Remarkably, one component of the putative laminin-binding dystrophin complex, β-dystroglycan, is expressed relatively late in retinal synaptogenesis, well after the dystrophins and laminins.

Laminins in the immunophotoreceptor Matrix (IPM) and retinal synaptic layers

The immunohistochemical studies reported herein, on rat and human retina, show laminin chains α3, a4, α5, β3, γ2, and γ3 surround inner segments, which are likely to reflect a location in the IPM. In the IPM, laminins may be important in maintaining the proper

mature environment for photoreceptors. The proposed role for laminins in the IPM, given that laminins are known to be involved in adhesion and that the IPM is thought to be important in retinal adhesion is in retinal adhesion. This is particularly likely for laminin 5, previously shown to be critical for dermal adhesion. It is described herein that photoreceptors can adhere to recombinant laminin β2 chain. It will now be possible to determine whether the heterotrimeric laminins in the IPM are involved in photoreceptor adhesion. Several laminin chains are also present in the mature plexiform layers. In particular, the laminin α3, α4, α5, β2, and γ3 chains were expressed in the outer plexiform layer in a location not likely to be associated with the vasculature.

Müller cells produce retinal laminins

The RNA encoding the laminin chains that are expressed in the mature neural retina are located in cells that span the retina. This location was consistent with the production of laminins by Müller cells. Müller cell cytoplasm is spread across the retina (Rasmussen (1972) *J Ultrustruct Res* 39:413-429) and RNA is distributed throughout these processes. In addition, it was shown that the distribution of RNA encoding CRALBP, in both rat and human retina (Libby et al. (1997) *supra*), is similar to that of the laminins: throughout the retina, in fibers coursing through the retinal cell layers. Together with the immunohistochemical data noted above, these data support a Müller cell source for laminins in the IPM and synaptic layers.

Laminins in the nervous system

In the peripheral nervous system, several cell types produce a variety of laminins. For example, the glial elements that wrap peripheral nerves, Schwann cells, have long been known to produce laminins, including at least one that contains the β2 chain. Conversely, laminins are thought to be important during Schwann cell differentiation. Also in the periphery, muscle cells appear to express several different laminin trimers on their surface, which are likely to be important in guiding innervating motor neurons to their synaptic targets in the muscle, as well as for stabilization of the synapse (Hunter et al. (1989) *Nature* 338:229-234). Importantly, one laminin chain, α2, has been shown to be involved in muscular dystrophies: mutations in α2 have been found in murine muscular dystrophies (Xu et al.,(1994) *Nut Genet* 8:297-302; Sunada et al. (1995) *Hum Mol Genet* 4:1055-1 061) and in some cases of a human congenital muscular dystrophy (Helbling-Leclerc et al. (1995) *Nut*

Genet 11:216-218).

Similarly, in the central nervous system, laminins are present in a variety of areas, particularly during development. The cellular sources of their component chains include all three major cell classes of the central nervous system: glia, neurons, and neuroglial progenitors. Glial cells, including astrocytes, Bergmann glia, and Müller cells, are thought to be a major source of laminins in the adult and developing CNS (Libby et al. (1997) *supra*). Neurons may also produce laminins (Sarthy and Fu (1990) *J Cell Biol* 110: 2099-2108). Finally, it has been shown that the retinal neuroglial progenitor may be a source of laminins during development (Libby et al. (1997) *supra*).

The ability of all of the major neural cell types of the CNS to produce laminins is consistent with the profusion of roles that have been proposed for laminins in the CNS. Most notably, as with laminins in the peripheral nervous system, laminins in the CNS are thought to be involved with axon outgrowth, based upon the laminins known roles in axon outgrowth in vitro (reviewed in Sanes (1989) *Ann Rev Neurosci* 12:491-516; Liesi (1990) *Experientia* 46:900-907) and their distribution along many developing pathways (see, for example, Cohen et al. (1987) *Dev Biol* 22: 407-418; and Liesi and Silver (1988) *Dev Biol* 130:774-785; Zhou (1990) *Dev Brain Res* 55:191-201). Laminins are also thought to be involved with neuronal differentiation in the CNS. For example, it has been shown that retinal laminins containing the β2 chain can promote rod photoreceptor differentiation in vitro (Hunter and Brunken (1997) *Mol Cell Neurosci* 10:7-15). In addition, it has been shown that β2 chain-containing laminins are vital during the differentiation of photoreceptors and their synapses *in vivo* (Libby et al. (1999) *supra*).

Novel laminin trimers in the central nervous system

Retinal basement membranes contain the laminin α1, α5, β1, β2, and γ1 chains. In contrast, the neural retina has a different complement of at least seven laminin chains: α3, α4, α5, β2, β3, γ2 and γ3. Of these, it is probable that the α3, β3, and γ2 chains assemble to form laminin 5, although laminin 5 has not been purified from retinal extracts. However, together with the biochemical data, the expression data suggest that there are at least two novel laminin trimers, laminin 14 (α4 β2 γ3) and laminin 15 (α5 β2 γ3), in the CNS. The apparent loss of the β3 chain in the adult outer plexiform layer suggests that there could be an additional novel laminin present, laminin 13 α3 β2 γ3).

Intriguingly, laminins 14 and 15 appear to be expressed in two locations: (1) within the inter-photoreceptor matrix, and (2) in the outer plexiform layer. The location of these laminins in the outer plexiform layer suggests that they may serve to stabilize retinal synapses, in a manner analogous to that suggested for β2-containing laminins, perhaps including laminin 11, at the neuromuscular junction. Laminins 14 and 15 are the first laminins that could be involved in formation or stabilization of synapses within the CNS. Moreover, these laminins are present at the same location as two components of the dystrophin complex--dystrophins and β-dystroglycan. The ability to examine the presence of all known laminin chains has allowed the demonstration that laminins are, in fact, associated with dystrophin complexes at central synapses, just as they are at the neuromuscular junction. However, as laminin expression precedes that of one component of the dystrophin complex (β-dystroglycan), it seems likely that it is not necessary to assemble the entire complex in order to stabilize laminins at the photoreceptor synapse.

The data describing the presence of laminin 5 during development suggests that this trimer may also be involved in retinal differentiation. This data, along with the data that suggest the presence of laminins 14 and 15 during development, provide support that laminins are critical components of the extracellular environment during differentiation of the nervous system.Laminins are also ubiquitously expressed in the vertebrate nervous system. All patents and references cited herein are incorporated in their entirety by reference. Other embodiments are within the following claims.

What is claimed:

1. A substantially pure preparation, comprising: a laminin wherein the laminin comprises laminin chain α5, laminin chain β2, and laminin chain γ3.

2. The preparation of claim 1, wherein the laminin is laminin-15.

3. A cell which comprises a nucleic acid encoding a laminin α5 chain, a nucleic acid encoding a laminin β2 chain, and a nucleic acid encoding a γ3 chain, wherein the cell is capable of producing laminin-15.

4. A composition comprising the protein preparation of claim 1 and a pharmaceutically acceptable carrier.

5. A method of isolating the preparation of claim 1, comprising:

obtaining a retinal tissue selected from the group consisting of retina interphotoreceptor matrix, retina outer plexiform layer, neural retina, Müller cell, a preparation of retinal neurons; and

isolating the preparation of claim 1.

6. A method of increasing retina immunophotoreceptor matrix stability, comprising: administering an effective amount of the preparation of claim 1.

7. A method of increasing the stability of one or more retina photoreceptor components, comprising: administering an effective amount of the preparation of claim 1.

8. The method of claim 7, wherein the retina photoreceptor component is an outer segment, inner segment, cell body, or synapse.

9. A method of increasing retina adhesion, comprising: administering an effective amount of the preparation of claim 1.

10. A method of treating a disorder associated with retina degeneration, comprising: administering an effective amount of the preparation of claim 1.

11. The method of claim 10, wherein the disorder is selected from a group consisting of: rod dystrophy, rod-cone dystrophy, retinitis pigmentosa, macular degeneration, and retinal detachment.

12. A method of increasing the stability of synapses, comprising: administering an effective amount of the preparation of claim 1.

13. The method of claim 12, wherein the synapses are selected from the group consisting of synapses of central nervous system and synapses of peripheral nervous system.

14. A method of stimulating a condition, comprising: administering an effective amount of a compound comprising the preparation of claim 1, wherein the condition is neuroregeneration, axon outgrowth, or formation of synapses.

15. The method of claim 14, wherein the synapses are selected from the group consisting of: synapses of central nervous system and synapses of peripheral nervous system.

16. A method of preparing an implant, comprising: coating the implant with the preparation of claim 1.

17. The method of claim 16, wherein the implant is selected from the group consisting of: an implantable tip, an implantable catheter, an artificial joint, a retinal implant, a timed releasing device, a neural cell growth guide, and an artificial tissue.

18. A method of increasing photosentivity, comprising: implanting a tip coated with an effective amount of the preparation of claim 1 into an eye.

19. The preparation of claim 1, wherein the laminin is recombinant.

20. A method for producing laminin 15, comprising: expressing laminin chain α5, laminin chain β2, and laminin chain γ3, and producing laminin 15.

21. The method of claim 20, wherein the laminin chain α5, laminin chain β2, and laminin chain γ3 are co-expressed in a cell.

22. The method of claim 21, wherein the laminin chain α5, laminin chain β2, and laminin chain γ3 are expressed in different cells.

Amino Acid Sequence and Nucleotide Sequence Encoding Murine Alpha5 Chain

DLYCKLVGGPVAGGDPNQTIQGQYCDICTAANSNKAHPVSNAIDGTERWWQSP
PLSRGLEYNEVNVTLDLGQVFHVAYVLIKFANSPRPDLWVLERSTDFGHTYQPW
QFFASSKRDCLERFGPRTLERITQDDDVICTTEYSRIVPLENGEIVVSLVNGRPGAL
NFSYSPLLRDFTKATNIRLRFLRTNTLLGHLMGKALRDPTVTRRYYYSIKDISIGG
RCVCHGHADVCDAKDPLDPFRLQCACQHNTCGGSCDRCCPGFNQQPWKPATTD
SANECQSCNCHGHAYDCYYDPEVDRRNASQNQDNVYQGGGVCLDCQHHTTGI
NCERCLPGFFRAPDQPLDSPHVCRPCDCESDFTDGTCEDLTGRCYCRPNFTGELC
AACAEGYTDFPHCYPLPSFPHNDTREQVLPAGQIVNCDCNAAGTQGNACRKDPR
LGRCVCKPNFRGAHCELCAPGFHGPSCHPCQCSSPGVANSLCDPESGQCMCRTG
FEGDRCDHCALGYFHFPLCQLCGCSPAGTLPEGCDEAGRCQCRPGFDGPHCDRC
LPGYHGYPDCHACACDPRGALDQQCGVGGLCHCRPGNTGATCQECSPGFYGFP
SCIPCHCSADGSLHTTCDPTTGQCRCRPRVTGLHCDMCVPGAYNFPYCEAGSCH
PAGLAPANPALPETQAPCMCRAHVEGPSCDRCKPGYWGLSASNPEGCTRCSCDP
RGTLGGVTECQGNGQCFCKAHVCGKTCAACKDGFFGLDYADYFGCRSCRCDV
GGALGQGCEPKTGACRCRPNTQGPTCSEPAKDHYLPDLHHMRLELEEAATPEGH
AVRFGFNPLEFENFSWRGYAHMMAIQPRIVARLNVTSPDLFRLVFRYVNRGSTS
VNGQISVREEGKLSSCTNCTEQSQPVAFPPSTEPAFVTVPQRGFGEPFVLNPGIWA
LLVEAEGVLLDYVVLLPSTYYEAALLQHRVTEACTYRPSALHSTENCLVYAHLP
LDGFPSAAGTEALCRHDNSLPRPCPTEQLSPSHPPLATCFGSDVDIQLEMAVPQPG
QYVLVVEYVGEDSHQEMGVAVHTPQRAPQQGVLNLHPCPYSSLCRSPARDTQH
HLAIFHLDSEASIRLTAEQAHFFLHSVTLVPVEEFSTEFVEPRVFCVSSHGTFNPSS
AACLASRFPKPPQPIILKDCQVLPLPPDLPLTQSQELSPGAPPEGPQPRPPTAVDPN
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Figure 1A

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Figure 1B

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Figure 1C

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Figure 1D

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Figure 1E

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10801 gtggggatgc gcggatgccc ctcaggaacc ctagcacttt ccaagcaggg aaaggcactg
10861 acccagaggc acgccaagcc cagtgtctcc ccgctacttt ggcattgagg gttcccagac
10921 cttggggttt gcctacactt tctatgaata acaagtcatt tctggtttac actgtctttt
10981 agaggaaaag gactctgtag aacagatat

Figure 1F

Amino Acid Sequence and Nucleotide Sequence Encoding Human Alpha5 Chain

SGVQLRTPRDLADLAAYTALKFYLQGPEPEPGQGTEDRFVMYMGSRQATGDYM
GVSLRDKKVHWVYQLGEAGPAVLSIDEDIGEQFAAVSLDRTLQFGHMSVTVER
QMIQETKGDTVAPGAEGLLNLRPDDFVFYVGGYPSTFTPPPLLRFPGYRGCIEMD
TLNEEVVSLYNFERTFQLDTAVDRPCARSKSTGDPWLTDGSYLDGTGFARISFDS
QISTTKRFEQELRLVSYSGVLFFLKQQSQFLCLAVQEGSLVLLYDFGAGLKKAVP
LQPPPPLTSASKAIQVFLLGGSRKRVLVRVERATVYSVEQDNDLELADAYYLGG
VPPDQLPPSLRWLFPTGGSVRGCVKGIKALGKYVDLKRLNTTGVSAGCTADLLV
GRAMTFHGHGFLRLALSNVAPLTGNVYSGFGFHSAQDSALLYYRASPDGLCQVS
LQQGRVSLQLLRTEVKTQAGFADGAPHYVAFYSNATGVWLYVDDQLQQMKPH
RGPPPELQPQPEGPPRLLLGGLPESGTIYNFSGCISNVFVQRLLGPQRVFDLQQNL
GSVNVSTGCAPALQAQTPGLGPRGLQATARKASRRSRQPARHPACMLPPHLRTT
RDSYQFGGSLSSHLEFVGILARHRNWPSLSMHVLPRSSRGLLLFTARLRPGSPSLA
LFLSNGHFVAQMEGLGTRLRAQSRQRSRPGRWHKVSVRWEKNRILLVTDGARA
WSQEGPHRQHQGAEHPQPHTLFVGGLPASSHSSKLPVTVGFSGCVKRLRLHGRP
LGAPTRMAGVTPCILGPLEAGLFFPGSGGVITLDLPGATLPDVGLELEVRPLAVT
GLIFHLGQARTPPYLQLQVTEKQVLLRADDGAGEFSTSVTRPSVLCDGQWHRLA
VMKSGNVLRLEVDAQSNHTVGPLLAAAAGAPAPLYLGGLPEPMAVQPWPPAYC
GCMRRLAVNRSPVAMTRSVEVHGAVGASGCPAA

1 tcaggggtgc agctgcgcac cccacgggat cttgccgacc ttgctgccta cactgccctc
61 aagttctacc tgcagggccc agagcctgag cctgggcagg gtaccgagga tcgctttgtg
121 atgtacatgg gcagccgcca ggccactggg gactacatgg gtgtgtctct gcgtgacaag
181 aaggtgcact gggtgtatca gctgggtgag gcgggccctg cagtcctaag catcgatgag
241 gacattgggg agcagttcgc agctgtcagc ctggacagga ctctccagtt tggccacatg
301 tccgtcacag tggagagaca gatgatccag gaaaccaagg gtgacacggt ggcccctggg
361 gcagaggggc tgctcaacct gcggccagac gacttcgtct tctacgtcgg ggggtacccc
421 agtaccttca cgcccctcc cctgcttcgc ttccccggct accggggctg catcgagatg
481 gacacgctga atgaggaggt ggtcagcctc tacaacttcg agaggacctt ccagctggac
541 acggctgtgg acaggccttg tgcccgctcc aagtcgaccg gggacccgtg gctcacggac
601 ggctcctacc tggacggcac cggcttcgcc cgcatcagct tcgacagtca gatcagcacc
661 accaagcgct tcgagcagga gctgcggctc gtgtcctaca gcggggtgct cttcttcctg
721 aagcagcaga gccagttcct gtgcttggcc gtgcaagaag gcagcctcgt gctgttgtat
781 gactttgggg ctggcctgaa aaaggccgtc ccactgcagc ccccaccgcc cctgacctcg
841 gccagcaagg cgatccaggt gttcctgctg gggggcagcc gcaagcgtgt gctggtgcgt
901 gtggagcggg ccacggtgta cagcgtggag caggacaatg atctggagct ggccgacgcc
961 tactacctgg ggggcgtgcc gcccgaccag ctgccccccga gcctgcgatg gctcttcccc
1021 accggaggct cagtccgtgg ctgcgtcaaa ggcatcaagg ccctgggcaa gtatgtggac
1081 ctcaagcggc tgaacacgac aggcgtgagc gccggctgca ccgccgacct gctggtgggg
1141 cgcgccatga ctttccatgg ccacggcttc cttcgcctgg cgctctcgaa cgtggcaccg
1201 ctcactggca acgtctactc cggcttcggc ttccacagcg cccaggacag tgccctgctc
1261 tactaccggg cgtccccgga tgggctatgc caggtgtccc tgcagcaggg ccgtgtgagc
1321 ctacagctcc tgaggactga agtgaaaact caagcgggct tcgccgatgg tgccccccat
1381 tacgtcgcct tctacagcaa tgccacggga gtctggctgt atgtcgatga ccagctccag
1441 cagatgaagc cccaccgggg accacccccc gagctccagc cgcagcctga ggggcccccg

Figur 2A

1501 aggctcctcc tgggaggcct gcctgagtct ggcaccattt acaacttcag tggctgcatc
1561 agcaacgtct tcgtgcagcg gctcctgggc ccacagcgcg tatttgatct gcagcagaac
1621 ctgggcagcg tcaatgtgag cacgggctgt gcacccgccc tgcaagccca gaccccgggc
1681 ctggggccta gaggactgca ggccaccgcc cggaaggcct cccgccgcag ccgtcagccc
1741 gcccggcatc ctgcctgcat gctgcccccа cacctcagga ccacccgaga ctcctaccag
1801 tttgggggtt ccctgtccag tcacctggag tttgtgggca tcctggcccg acataggaac
1861 tggcccagtc tctccatgca cgtcctcccg cgaagctccc gaggcctcct cctcttcact
1921 gcccgtctga ggcccggcag ccccтccctg gcgctcttcc tgagcaatgg ccacttcgtt
1981 gcacagatgg aaggcctcgg gactcggctc cgcgcccaga gccgccagcg ctcccggcct
2041 ggccgctggc acaaggtctc cgtgcgctgg gagaagaacc ggatcctgct ggtgacggac
2101 ggggcccggg cctggagcca ggaggggccg caccggcagc accagggggc agagcacccc
2161 cagccccaca ccctctttgt gggcggcctc ccggccagca gccacagctc caaacttccg
2221 gtgaccgtcg ggttcagcgg ctgtgtgaag agactgaggc tgcacgggag gcccctgggg
2281 gcccccacac ggatggcagg ggtcacaccc tgcatcttgg gcccccctgga ggcgggcctg
2341 ttcttcccag gcagcggggg agttatcact ttagacctcc caggagctac actgcctgat
2401 gtgggcctgg aactggaggt gcggcccctg gcagtcaccg gactgatctt ccacttgggc
2461 caggcccgga cgccccccta cttgcagttg caggtgaccg agaagcaagt cctgctgcgg
2521 gcggatgacg gagcagggga gttctccacg tcagtgaccc gcccctcagt gctgtgtgat
2581 ggccagtggc accggctagc ggtgatgaaa agcgggaatg tgctccggct ggaggtggac
2641 gcgcagagca accacaccgt gggcccccttg ctggcggctg cagctggtgc cccagcccct
2701 ctgtacctcg ggggcctgcc tgagcccatg gccgtgcagc cctggccccc cgcctactgc
2761 ggctgcatga ggaggctggc ggtgaaccgg tcccccgtcg ccatgactcg ctctgtggag
2821 gtccacgggg cagtgggggc cagtggctgc ccagccgcct aggacacagc caacccccggc
2881 ccctggtcag gcccctgcag ctgcctcaca ccgcccccttg tgctcgcctc ataggtgtct
2941 atttggactc taagctctac gggtgacaga tcttgtttct gaagatggtt taagttatag
3001 cttcttaaac gaaagaataa aatactgcaa aatgttttta tatttggccc ttccacccat
3061 ttttaattgt gagagatttg tcaccaatca tcactggttc ctccttaaaa attaaaaagt
3121 aacttctgtg taaaaaaaaa a

Figure 2B

Amino Acid Sequence and Nucleotide Sequence Encoding Murine Beta2 Chain

MEWASGEPGRGRQGQPLPWELRLGLLLSVLAATLAQAPSLDVPGCSRGS
CYPATGDLLVGRADRLTASSTCGLHSPQPYCIVSHLQDEKKCFLCDSRRP
FSARDNPNSHRIQNVVTSFAPQRRTAWWQSENGVPMVTIQLDLEAEFHFT
HLIMTFKTFRPAAMLVERSADFGRTWHVYRYFSYDCGADFPGIPLAPPRR
WDDVVCESRYSEIEPSTEGEVIYRVLDPAIPIPDPYSSRIQNLLKITNLRVNL
TRLHTLGDNLLDPRREIREKYYYALYELVIRGNCFCYGHASQCAPAPGAP
AHAEGMVHGACICKHNTRGLNCEQCQDFYQDLPWHPAEDGHTHACRKC
ECNGHTHSCHFDMAVYLASGNVSGGVCDGCQHNTAGRHCEFCRPFFYR
DPTKDMRDPAVCRPCDCDPMGSQDGGRCDSHDDPVLGLVSGQCRCKEH
VVGTRCQQCRDGFFGLSASDPRGCQRCQCNSRGTVPGSSPCDSSSGTCFC
KRLVTGHGCDRCLPGHWGLSHDLLGCRPCDCDVGGALDPQCDEATGQC
PCRQHMIGRRCEQVQPGYFRPFLDHLTWEAEAAQGQVLEVVERLVTNRE
TPSWTGPGFVRLREGQEVEFLVTSLPRAMDYDLLLRWEPQVPEQWAELE
LMVQRPGPVSAHSPCGHVLPKDDRIQGMLHPNTRVLVFPRPVCLEPGISY
KLKLKLIGTGGRAQPETSYSGLLIDSLVLQPHVLVLEMFSGGDAAALERR
TTFERYRCHEEGLMPSKAPLSETCAPLLISVSALIYNGALPCQCDPQGSLSS
ECSPHGGQCRCKPGVVGRRCDVCATGYYGFGPAGCQACQCSPDGALSA
LCEGTSGQCPCRPGAFGLRCDHCQRGQWGFPNCRPCVCNGRADECDTHT
GACLGCRDYTGGEHCERCIAGFHGDPRLPYGGQCRPCPCPEGPGSQRHFA
TSCHRDGYSQQIVCHCRAGYTGLRCEACAPGPFGDPSKPGGRCQLCECSG
NIDPMDPDACDPHTGQCLRCLHNTEGPHCGYCKPGFHGQAARQSCHRCT
CNLLGTDPRRCPSTDLCHCDPSTGQCPCLPHVQGLNCDHCAPNFWNFTSG
RGCQPCACHPSRARGPTCNEFTGQCHCHAGFGGRTCSECQELYWGDPGL
QCRACDCDPRGIDKPQCHRSTGHCSCRPGVSGVRCDQCARGFSGVFPAC
HPCHACFGDWDRVVQDLAARTRRLEQWAQELQQTGVLGAFESSFLNMQ
GKLGMVQAIMSARNASAASTAKLVEATEGLRHEIGKTTERLTQLEAELTA
VQDENFNANHALSGLERDGFALNLTLRQLDQHLEILKHSNFLGAYDSIRH
AHSQSTEAERRANASTFAVPSPVSNSADTRRRTEVLMGAQKENFNRQHL
ANQQALGRLSAHAHTLSLTGINELVCGAPGDAPCATSPCGGAGCRDEDG
QPRCGGLGCSGAAAPADLALGRARHSQAELQRALVEGGGILSRVSETRR
QAEAAQQRAQAALDKANASRGQVEQANQELRELIQNVKDFLSQEGADP
DSIEMVATRVLDISIPASPEQIQRLASEIAERVRSLADVDTILAHTMGDVRR
AEQLLQDAHRARSRAEGERQKAETVQAALEEAQRAQGAAQGAIRGAVV
DTQNTEQTLQRVQERMAGAEKSLNSAGERARQLDALLEALKLKRAGNSL
AASTAEETAGSAQSRAREAEKQLREQVGDQYQTVRALAERKAEGVLAA
QARAEQLRDEARDLLQAAQDKLQRLQELEGTYEENERALEGKAAQLDG
LEARMRSVLQAINLQVQIYNTCQ

1 aaagggcctc gagcttccaa gtaatctttg cttgatctcc aagagtctgt catagcgtgg
61 cactcaaacg aagccgtacg acctgaacca acctcttccg cctgttgtcc aggggtctgg
121 gtggnnngcg cctagtgggt gcgcgcattc caaccctcgc tcccgggctg ccaggcgact
181 ggaaagtccg gcgtggataa atagtcacaa gattcggatt cacttgttgc tggtggtcca
241 gagtctgtca cccagaaccc atcctctggg taactgagta gccacagccc attttaatca

Figure 3A

301 ggaaacaggc aacctttctc gcaacccatt tgctggagtg cttatggacg gtcgagttcc
361 tcggagttct gtttcaggca gtagtgcgtg gcctttccag tatctccgag agctcagtcc
421 tagtctatcc tttgggcgtc ctaaaccttt ccacaggtac aatagaattc tagcttgcac
481 ctttcccatc catctcccga ctgatgctgt aaccctggga gccccgcggc tgatttgtgg
541 tttccatagt gacaccagga caaaggccat aagctccttc catctgcctt cctgatacac
601 aaagatcaca aacctctcga tttacctctg ccacccgcca actccacgag ccctcttcct
661 gtcccctgaa tgccatgctt gccagcaacc cctggttcac atcgggactt aagggatccg
721 atgaagatat gtggaccagg atgctctgtc tttgagcagc ctactctaat ttctttttgg
781 atgctccctt ttagttcctc gaactaagct gcttctttgc taagtacaca tctgctaaat
841 aaacttcagc ttaaaaagaa agtggatgaa gtaaccaaag tctggttctt aggatgaggg
901 ttgtctgcag cgggcagggg tatggtaggg grtggggtgc tatcctcagt tataatccta
961 ttttagatcc actccgtgtt ttacttcctt cccttgcttt ccaactttac tcaaagtcgt
1021 cagagtctct cagattgtgg aggagtgact gctaggtccg accctggaca gattgagggc
1081 ctggagggac accagcccag tacccacacg gtcgggtcag catcagcccc aagggaggtg
1141 gtgggctttc gtctgtggac tctttatctc tctttatctc tattttactt ttcttcagga
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1441 ggccccgtcc ttggatgtac ctggctgttc tcgaggaagc tgctatccag ccaccggtga
1501 cctgttggtg ggccgtgcgg acagactgac ggcctcatcc acgtgtggct tgcatagccc
1561 tcaaccctac tgtattgtca gtcacctgca ggtgcttctg gggccccaga ggagagggct
1621 gggtcagggt ggggtcggcc ccagctaagg tacctatcct acactccacc caatccagga
1681 cgaaaagaag tgtttcctgt gtgactcccg acgtcccttc tctgctcgag acaacccaaa
1741 tagtcatcgg atccagaatg tagtcaccag ctttgcgcca caacgccgga cggcctggtg
1801 gcaatcggag aacggtgagc ccctgagtag gtcatcagga tgactagggc ttgtaaccag
1861 taaccgcaga accttgaccc cctattcctg ctgcaggggt tccaatggtc accatccaac
1921 tggacctgga agctgagttt catttcaccc acctcattat gacgttcaag gtgcctgtgc
1981 gtcagcgaac ccgcctgatt ttgctttgct tctcagtacc ccctaacaga gtcctagctc
2041 tacaacgaag cttccctgag ctcctgtgtt gtgctctatg tgtgaagcat ggtcacgtcc
2101 tgcatggctt ccatagttga acacctctgc acatgctctc ttgtgcccca ttcctaggct
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2221 tcctccctgg tctgatcatg gtctggcttt cctagtcttt cccatgtgtc tgagatgctc
2281 agcagtgatc atgactaagc agagctctcc gtaacctagg ctggactgaa gtctggttcc
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2581 ctagtggggt ctgtgatgga cgtggacgag gttcattatc tgtggacttc ttgccctgct
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2701 ttcagagtga gtgttctact atggacattg gcacagtctc agtgtccgga tgggactatt
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2881 ctggtgggga cgaggcaaca aggggccacc tgcttagttg gacgagaccc tcttcccttt
2941 cttagacctg ttgaagatca ccaacctacg agtgaactta acccggcttc acacactggg
3001 agacaacttg cttgacccac ggagggagat ccgggaaaaa tactattatg ctctctatga

Figure 3B

3061 acttgtcatc cgtggcaact gcttctgcta tggccacgcc tcacagtgtg cgcctgcacc
3121 aggggcgccg gcccatgctg agggcatggt aagggacttc ggatgactgg aacagggttg
3181 ccggggaggg acaggcattt ctagatggtg ccgtcaacct cccctcgtat ctgcacaggt
3241 acacggagcc tgtatctgca agcacaatac tcgtggactc aactgtgagc agtgtcagga
3301 tttctatcag gaccttcccc ggcaccctgc agaggacggc catactcacg cctgtcggag
3361 tgagtgagac acagaactct aaccgggctg tgctctgggt gagccaaaaa gctagttggt
3421 caagccctaa atacctaggc ytttgtctga agggtatcag gccttgatgg cctcaaccca
3481 tgtgctctgc tacagtccaa agttggagct tgaagctaag ctgcaccaca aattctagct
3541 atggtaccat aggctgatga tactagcccc actcgcgtgt ccttacctag gacctggttt
3601 ccaattggtc tttgccttct ctccagagtg tgagtgcaac gggcatactc atagctgcca
3661 ctttgacatg gctgtctacc tggcatctgg aaatgtaagt ggaggcgtat gcgatgggtg
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3781 caccaaggac atgcgggacc cagctgtgtg ccgtcgtaag gctgggattg ggcatgaggc
3841 tgantctcag aactagaact aggaacgtgg attatatgac gttcccagga ttggtgtggt
3901 cagggcttgg ggtagaacca gaacagggaa agggaaggct caggatggtc actgcgatgg
3961 ggtgacttta tactctcctt tttctcagct tgtgactgtg accctatggg ttctcaagat
4021 ggtggtcgct gtgattctca tgatgaccct gtgctaggac tggtctcagg ccagtgtcgc
4081 tgcaaagaac acgtggttgg cactcgctgc cagcaatgcc gtgatggctt ctttggactt
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4321 cttgtgactc cagtagtgga acctgtttct gcaagcgtct ggtgaccgga catggctgtg
4381 accgctgtct ggtacgactg agggatctgg ggtcctggga tcctgggttt gttctcaaag
4441 cacatgggca aagtccagtg ggtggacact gagagcctag agtctagtcc tggaagacaa
4501 gcgtctggtc tggcaggtca agggtctaga ccagtggtct ggggctttgc attcaccagt
4561 ccaagtggta aattgctgac tacctggtgg gtggcaagga ggtctgttcc tggcttccag
4621 caattccttt tccctagcct ggccactggg gcctgagcca tgacctgctg ggctgccgtc
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4861 tgcagcctgg ctacttccgg ccttttctgg accatttaac ctgggaggct gaggctgccc
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5341 cctctctccc caggtccctg agcaatgggc agagctggaa ctgatggtgc agcgtccggg
5401 gcctgtgtct gctcacagtc cgtgcgggca tgtgctgcct aaggatgacc gcattcaggg
5461 gatgcttcac ccaaacacca ggtgaggcgg nggggtaagga ttgcccacag acctcctgaa
5521 agactgacat tgcgctgtgt tgttccttct ttaagtccct cctcctggct gctgttcgtc
5581 aggtccatgg ctgtgactca caggaaagac atagataaca catggcctgc ttcctcaagg
5641 gtataagttt cagaaggcaa gacattaatt ggtctgttac tccgaaacag ccttatgatg
5701 gtgacagttg cagtggcgta agatatgtaa ctggactagt taaggttttg ttacatttta
5761 gaagtaatta tttcctgtat ctttttcctc actactctct gctcttctct tctcttctct

Figure 3C

5821 tctcttctct tctcttctct actcttctct tctctactct actagtctaa acttatcttc
5881 tgctcttacc tctctctctc tctcaacctg agacagggtt tctctgtata gccccagggt
5941 gtcctggaac tcactac

Figure 3D

Amino Acid Sequence and Nucleotide Sequence Encoding Human Beta2 Chain

MELTSTERGRGQPLPWELRLPLLLSVLAATLAQAPAPDVPGCSRGSCYPATADLL
VGRADRLTASSTCGLNGRQPYCIVSHLQDEKKCFLCDSRRPFSARDNPHTHRIQN
VVTSFAPQRRAAWWQSQNGIPAVTIQLDLEAEFHFTHLIMTFKTFRPAAMLVERS
ADFGRTWHVYRYFSYHCGADFPGVPLAPPRHWDDVVCESRYSEIEPSTEGEVIY
RVLDPAIPIPDPYSSRIQNLLKITNLRVNLTRLHTLGDNLLDPRREIREKYYYALYE
LVVRGNCFCYGHASECAPAPGAPAHAEGMVHGACICKHNTRGLNCEQCQDFYR
DLPWRPAEDGHSHACRKCDRHGHTHSCHFDMAVYLGSGNVSGGVCDGCQHNT
AWRHCELCRPFFYRDPTKDLRDPAVCRSCDCDPMGSQDGGRCDSHDDPALGLV
SGQCRCKEHVVGTRCQQCRDGFFGLSISDPSGCRRCQCNARGTVPGSTPCDPNS
GSCYCKRLVTGRGCDRCLPGHWGLSLDLLGCRPCDCDVGGALDPQCDEGTGQC
HCRQHMVGRRCEQVQPGYFRPFLDHLIWEAENTRGQVLDVVERLVTPGETPSW
TGSGFVRLQEGQTLEFLVASVPNAMDYDLLLRLEPQVPEQWAELELIVQRPGPV
PAHSLCGHLVPRDDRIQGTLQPHARYLIFPNPVCLEPGISYKLHLKLVRTGGSAQP
ETPYSGPGLLIDSLVLLPRVLVLEMFSGGDAAALERQATFERYQCHEEGLVPSKT
SPSEACAPLLISLSTLIYNGALPCQCNPQGSLSSECNPHGGQCLCKPGVVGRRCDT
CAPGYYGFGPTGCQACQCSPRGALSSLCERTSGQCLCRTGAFGLRCDACQRGQ
WGFPSCRPCVCNGHADECNTHTGACLGCRDLTGGEHCERCIAGFHGDPRLPYG
AQCRPCPCPEGPGSQRHFATSCHQDEYSQQIVCHCRAGYTGLRCEACAPGQFGD
PSRPGGRCQLCECSGNIDPMDPDACDPHPGQCLRCLHHTEGPHCAHSKPGFHGQ
AARQSCHRCTCNLLGTNPQQCPSPDQCHCDPSSGQCPCLPNVQALAVDRCAPNF
WNLTSGHGCQPCACLPSPEEGPTCNEFTGQCHCLCGFGGRTCSECQELHWGDPG
LQCHACDCDSRGIDTPQCHRFTGHCTCRPGVSGVRCDQCARGFSGIFPACHPCH
ACFGDWDRVVQDLAARTQRLEQRAQELQQTGVLGAFESSFWHMQEKLGIVQGI
VGARNTSAASTAQLVEATEELRREIGEATEHLTQLEADLTDVQDENFNANHALS
GLERDRLALNLTLRQLDQHLDLLKHSNFLGAYDSIRHAHSQSAEAERRANTSAL
AVPSPVSNSASARHRTEALMDAQKEDFNSKHMANQRALGKLSAHTHTLSLTDIN
ELVCGAQGLHHDRTSPCGGAGCRDEDGQPRCGGLSCNGAAATADLALGRARHT
QAELQRALAEGGSILSRVAETRRQASEAQQRAQAALDKANASRGQVEQANQEL
QELIQSVKDFLNQEGADPDSIEMVATRVLELSIPASAEQIQHLAGAIAERVRSLAD
VDAILARTVGDVRRAEQLLQDARRARSWAEDEKQKAETVQAALEEAQRAQGIA
QGAIRGAVADTRDTEQTLYQVQERMAGAERALSSAGERARQLDALLEALKLKR
AGNSLAASTAEETAGSAQGRAQEAEQLLRGPLGDQYQTVKALAERKAQGVLAA
QARAEQLPDEARDLLQAAQDKLQRLQELEGTYEENERALESKAAQLDGLEARM
RSVLQAINLQVQIYNTCQ

1 ccgcccggtg ttgcgctcct tcccagaatc cgctccggcc tttccttcct gccgcgattc
61 ccaactttgc tcaaagtcgc cggactctaa gctgtcggag ggaccgctgg acagacctgg
121 gaactgacag agggcctgga gggaaatagg ccaaagaccc acaggatgga gctgacctca
181 accgaaagag ggaggggaca gcctctgccc tgggaacttc gactgcccct actgctaagc
241 gtgctggctg ccacactggc acaggcccct gccccggatg tccctggctg ttccagggga
301 agctgctacc ccgccacggc cgacctgctg gtgggccgag ctgacagact gactgcctca
361 tccacttgtg gcctgaatgg ccgccagccc tactgcatcg tcagtcacct gcaggacgaa
421 aagaagtgct tcctttgtga ctcccggcgc cccttctctg ctagagacaa cccacacacc

Figure 4A

481 catcgcatcc agaatgtagt caccagcttt gcaccacagc ggcgggcagc ttggtggcag
541 tcacagaatg gtatccctgc ggtcaccatc cagctggacc tggaggctga gtttcatttc
601 acacacctca ttatgacctt caagacattt cgccctgctg ccatgctggt cgaacgctca
661 gcagactttg gccgcacctg gcatgtgtac cgatatttct cctatcactg tggggctgac
721 ttcccaggag tcccactagc acccccacgg cactgggatg atgtagtctg tgagtcccgc
781 tactcagaga ttgagccatc cactgaaggc gaggtcatct atcgtgtgct ggaccctgcc
841 atccctatcc cagacccctа cagctcacgg attcagaacc tgttgaagat caccaaccta
901 cgggtgaacc tgactcgtct acacacgttg ggagacaacc tactcgaccc acggagggag
961 atccgagaga agtactacta tgccctctat gagctggttg tacgtggcaa ctgcttctgc
1021 tacggacacg cctcagagtg tgcacccgcc ccaggggcac cagcccatgc tgagggcatg
1081 gtgcacggag cttgcatctg caaacacaac acacgtggcc tcaactgcga gcagtgtcag
1141 gatttctatc gtgacctgcc ctggcgtccg gctgaggacg gccatagtca tgcctgtagg
1201 aagtgtgatc ggcatgggca cacccacagc tgccacttcg acatggccgt atacctcgga
1261 tctggcaatg tgagtggagg tgtgtgtgat ggatgtcagc ataacacagc gtggcgccac
1321 tgtgagctct gtcggccctt cttctaccgt gacccaacca aggacctgcg ggatccggct
1381 gtgtgccgct cctgtgattg tgaccccatg ggttctcaag acggtggtcg ctgtgattcc
1441 catgatgacc ctgcactggg actggtctcc ggccagtgtc gctgcaaaga acacgtggtg
1501 ggcactcgct gccagcaatg ccgtgatggc ttctttgggc tcagcatcag tgacccgtct
1561 gggtgccggc gatgtcaatg taatgcacgg ggcacagtgc ctgggagcac tccttgtgac
1621 cccaacagtg gatcctgtta ctgcaaacgt ctagtgactg gacgtggatg tgaccgctgc
1681 ctgcctggcc actggggcct gagcctcgac ctgctcggct gccgcccctg tgactgcgac
1741 gtgggtggtg ctttggatcc ccagtgtgat gagggcacag gtcaatgcca ctgccgccag
1801 cacatggttg ggcgacgctg tgagcaggtg caacctggct acttccggcc cttcctggac
1861 cacctaattt gggaggctga gaacacccga gggcaggtgc tcgatgtggt ggagcgcctg
1921 gtgacccccg gggaaactcc atcctggact ggctcaggct tcgtgcgact acaggaaggt
1981 cagaccctgg agttcctggt ggcctctgtg ccgaacgcga tggactatga cctgctgctg
2041 cgcttagagc cccaggtccc tgagcaatgg gcagagttgg aactgattgt gcagcgtcca
2101 gggcctgtgc ctgcccacag cctgtgtggg catttggtgc ccagggatga tcgcatccaa
2161 gggactctgc aaccacatgc caggtacttg atatttccta atcctgtctg ccttgagcct
2221 ggtatctcct acaagctgca tctgaagctg gtacggacag ggggaagtgc ccagcctgag
2281 actccctact ctggacctgg cctgctcatt gactcgctgg tgctgctgcc ccgtgtcctg
2341 gtgctagaga tgtttagtgg gggtgatgct gctgccctgg agcgccaggc caccttтgaa
2401 cgctaccaat gccatgagga gggtctggtg cccagcaaga cttctccctc tgaggcctgc
2461 gcacccctcc tcatcagcct gtccacccтc atctacaatg gtgccctgcc atgtcagtgc
2521 aaccctcaag gttcactgag ttctgagtgc aaccctcatg gtggtcagtg cctgtgcaag
2581 cctggagtgg ttgggcgccg ctgtgacacg tgtgcccctg gctactatgg ctttggcccc
2641 acaggctgtc aagcctgcca gtgcagccca cgaggggcac tcagcagtct ctgtgaaagg
2701 accagtgggc aatgtctctg tcgaactggt gcctttgggc ttcgctgtga cgcctgccag
2761 cgtggccagt ggggattccc tagctgccgg ccatgtgtct gcaatgggca tgcagatgag
2821 tgcaacaccc acacaggcgc ttgcctgggc tgccgtgatc tcacaggggg tgagcactgt
2881 gaaaggtgca ttgctggttt ccacggggac ccacggctgc catatggggc gcagtgccgg
2941 ccctgtccct gtcctgaagg ccctgggagc caacggcact ttgctacttc ttgccaccag
3001 gatgaatatt cccagcagat tgtgtgccac tgccgggcag gctatacggg gctgcgatgt
3061 gaagcttgtg cccctgggca gtttggggac ccatcaaggc caggtggccg gtgccaactg
3121 tgtgagtgca gtgggaacat tgacccaatg gatcctgatg cctgtgaccc acaccccggg
3181 caatgcctgc gctgtttaca ccacacagag ggtccacact gtgcccactc gaagcctggc

Figure 4B

3241 ttccatggcc aggctgcccg gcagagctgt caccgctgca catgcaacct gctgggcaca
3301 aatccgcagc agtgcccatc tcctgaccag tgccactgtg atccaagcag tgggcagtgc
3361 ccatgcctcc ccaatgtcca ggccctagct gtagaccgct gtgcccccaa cttctggaac
3421 ctcaccagtg gccatggttg ccagccttgt gcctgcctcc caagcccgga agaaggcccc
3481 acctgcaacg agttcacagg gcagtgccac tgcctgtgcg gctttggagg gcggacttgt
3541 tctgagtgcc aagagctcca ctggggagac cctgggttgc agtgccatgc ctgtgattgt
3601 gactctcgtg gaatagatac acctcagtgt caccgcttca caggtcactg cacgtgccgc
3661 ccaggggtgt ctggtgtgcg ctgtgaccag tgtgcccgtg gcttctcagg aatctttcct
3721 gcctgccatc cctgccatgc atgcttcggg gattgggacc gagtggtgca ggacttggca
3781 gcccgtacac agcgcctaga gcagcgggcg caggagttgc aacagacggg tgtgctgggt
3841 gcctttgaga gcagcttctg gcacatgcag gagaagctgg gcattgtgca gggcatcgta
3901 ggtgcccgca acacctcagc cgcctccact gcacagcttg tggaggccac agaggagctg
3961 cggcgtgaaa ttggggaggc cactgagcac ctgactcagc tcgaggcaga cctgacagat
4021 gtgcaagatg agaacttcaa tgccaaccat gcactaagtg gtctggagcg agataggctt
4081 gcacttaatc tcacactgcg gcagctcgac cagcatcttg acttgctcaa acattcaaac
4141 ttcctgggtg cctatgacag catccggcat gcccatagcc agtctgcaga ggcagaacgt
4201 cgtgccaata cctcagccct ggcagtacct agccctgtga gcaactcggc aagtgctcgg
4261 catcggacag aggcactgat ggatgctcag aaggaggact tcaacagcaa acacatggcc
4321 aaccagcggg cacttggcaa gctctctgcc catacccaca ccctgagcct gacagacata
4381 aatgagctgg tgtgtggggc ccagggattg catcatgatc gtacaagccc ttgtgggggt
4441 gccggctgtc gagatgagga tgggcagccg cgctgtgggg gcctcagctg caatggggca
4501 gcggctacag cagacctagc actgggccgg gcccggcaca cacaggcaga gctgcagcgg
4561 gcactggcag aaggtggtag catcctcagc agagtggctg agactcgtcg gcaggcaagc
4621 gaggcacagc agcgggccca ggcagccctg gacaaggcta atgcttccag gggacaggtg
4681 gaacaggcca accaggaact tcaagaactt atccagagtg tgaaggactt cctcaaccag
4741 gaggggggctg atcctgatag cattgaaatg gtggccacac gggtgctaga gctctccatc
4801 ccagcttcag ctgagcagat ccagcacctg gcgggcgcga ttgcagagcg agtccggagc
4861 ctggcagatg tggatgcgat cctggcacgt actgtaggag atgtgcgtcg tgccgagcag
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4981 acagtacagg cagcactgga ggaggcccag cgggcacagg gtattgccca gggtgccatc
5041 cgggggggcag tggctgacac acgggacaca gagcagaccc tgtaccaggt acaggagagg
5101 atggcaggtg cagagcgggc actgagctct gcaggtgaaa gggctcggca gttggatgct
5161 ctcctggagg ctctgaaatt gaaacgggca ggaaatagtc tggcagcctc tacagcagaa
5221 gaaacggcag gcagtgccca gggtcgtgcc caggaggctg agcagctgct acgcggtcct
5281 ctgggtgatc agtaccagac ggtgaaggcc ctagctgagc gcaaggccca aggtgtgctg
5341 gctgcacagg caagggcaga acaactgccg gatgaggctc gggacctgtt gcaagccgct
5401 caggacaagc tgcagcggct acaggaattg gaaggcacct atgaggaaaa tgagcgggca
5461 ctggagagta aggcagccca gttggacggg ttggaggcca ggatgcgcag cgtgcttcaa
5521 gccatcaact tgcaggtgca gatctacaac acctgccagt gacccctgcc caaggcctac
5581 cccagttcct agcactgccc cacatgcatg tctgcctatg cactgaagag ctcttggccc
5641 ggcagggccc ccaataaacc agtgtgaacc cccaaaaaaa aaa

Figure 4C

Amino Acid Sequence and Nucleotide Sequence Encoding Gamma3 Chain

MAAAALLLGLALLAPRAAGAGMGACYDGAGRPQRCLPVFENAAFGRLAQASH
TCGSPPEDFCPHVGAAGAGAHCQRCDAADPQRHHNASYLTDFHSQDESTWWQS
PSMAFGVQYPTSVNITLRLGKAYEITYVRLKFHTSRPESFAIYKRSRADGPWEPY
QFYSASCQKTYGRPEGQYLRPGEDERVAFCTSEFSDISPLSGGNVAFSTLEGRPSA
YNFEESPGLQEWVTSTELLISLDRLNTFGDDIFKDPKVLQSYYYAVSDFSVGGRC
KCNGHASECGPDVAGQLACRCQHNTTGTDCERCLPFFQDRPWARGTAEAAHEC
LPCNCSGRSEECTFDRELFRSTGHGGRCHHCRDHTAGPHCERCQENFYHWDPR
MPCQPCDCQSAGSLHLQCDDTGTCACKPTVTGWKCDRCLPGFHSLSEGGCRPCT
CNPAGSLDTCDPRSGRCPCKENVEGNLCDRCRPGTFNLQPHNPAGCSSCFCYGH
SKVCASTAQFQVHHILSDFHQGAEGWWARSVGGSEHSPQWSPNGVLLSPEDEEE
LTAPGKFLGDQRFSYGQPLILTFRVPPGDSPLPVQLRLEGTGLALSLRHSSLSGPQ
DARASQGGRAQVPLQETSEDVAPPLPPFHFQRLLANLTSLRLRVSPGPSPAGPVF
LTEVRLTSARPGLSPPASWVEICSCPTGYTGQFCESCAPGYKREMPQGGPYASCV
PCTCNQHGTCDPNTGICVCSHHTEGPSCERCLPGFYGNPFAGQADDCQPCPCPGQ
SACTTIPESGEVVCTHCPPGQRGRRCEVCDDGFFGDPLGLFGHPQPCHQCQCSGN
VDPNAVGNCDPLSGHCLRCLHNTTGDHCEHCQEGFYGSALAPRPADKCMPCSC
HPQGSVSEQMPCDPVTGQCSCLPHVTARDCSRCYPGFFDLQPGRGCRSCKCHPL
GSQEDQCHPKTGQCTCRPGVTGQACDRCQLGFFGSSIKGCRACRCSPLGAASAQ
CHYNGTCVCRPGFEGYKCDRCHYNFFLTADGTHCQQCPSCYALVKEETAKLKA
RLTLTEGWLQGSDCGSPWGPLDILLGEAPRGDVYQGHHLLPGAREAFLEQMMG
LEGAVKAAREQLQRLNKGARCAQAGSQKTCTQLADLEAVLESSEEEILHAAAIL
ASLEIPQEGPSQPTKWSHLAIEARALARSHRDTATKIAATAWRALLASNTSYALL
WNLLEGRVALETQRDLEDRYQEVQAAQKALRTAVAEVLPEAESVLATVQQVG
ADTAPYLALLASPGALPQKSRAEDLGLKAKALEKTVASWQHMATEAARTLQTA
AQATLRQTEPLTMARSRLTATFASQLHQGARAALTQASSSVQAATVTVMGART
LLADLEGMKLQFPRPKDQAALQRKADSVSDRLLADTRKKTKQAERMLGNAAPL
SSSAKKKGREAEVLAKDSAKLAKALLRERKQAHRRASRLTSQTQATLQQASQQ
VLASEARRQELEEAERVGAGLSEMEQQIRESRISLEKDIETLSELLARLGSLDTHQ
APAQALNETQWALERLRLQLGSPGSLQRKLSLLEQESQQQELQIQGFESDLAEIR
ADKQNLEAILHSLPENCASWQ

1 ccccgcaggg gaaggcgggt cctggcggcc agcgcgcggt ccgcgcccac cctagccgac
61 ggggccggca gagcgcgcgg cgtcggtgcc cttgaccatg gcggcggctg cgcttctgct
121 ggggctggcg ctgctggcac cgcgggcggc cggcgcgggc atgggcgcgt gctatgacgg
181 cgcagggcgc ccgcagcgct gcctgccggt gttcgagaac gcggcgtttg ggcggctcgc
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421 ggccttcggc gtgcagtacc ccacctcggt caacatcacc ctccgcctag ggaaggctta
481 tgagatcacg tatgtgaggc tgaagttcca caccagtcgc cctgagagct ttgccatcta
541 caagcgcagc cgcgccgacg gcccatggga gccctaccag ttctacagcg cctcctgcca
601 gaagacctac ggccggcccg agggccagta cctgcgcccc ggcgaggacg agcgcgtggc
661 cttctgcacc tctgagttca gcgacatctc cccgctgagt ggcggcaacg tggccttctc
721 caccctggag ggccggccca gcgcctacaa cttcgaggag agccctgggc tgcaggagtg

Figure 5A

781 ggtcaccagc accgaactcc tcatctctct agaccggctc aacacgtttg gggacgacat
841 cttcaaggac cccaaggtgc tccagtccta ctattatgcc gtgtccgact tctctgtggg
901 cggcaggtgc aagtgcaacg ggcatgccag cgagtgcggc cccgacgtgg caggccagtt
961 ggcctgccgg tgccagcaca acaccaccgg cacagactgt gagcgctgcc tgcccttctt
1021 ccaggaccgc ccgtgggccc ggggcaccgc cgaggctgcc cacgagtgtc tgccctgcaa
1081 ctgcagtggc cgctccgagg aatgcacgtt tgatcgggag ctcttccgca gcacaggcca
1141 cggcgggcgc tgtcaccact gccgtgacca cacagctggg ccacactgtg agcgctgtca
1201 ggagaatttc tatcactggg acccgcggat gccatgccag ccctgtgact gccagtcggc
1261 aggctcccta cacctccagt gcgatgacac aggcacctgc gcctgcaagc ccacagtgac
1321 tggctggaag tgtgaccgct gtctgcccgg gttccactcg ctcagtgagg gaggctgcag
1381 accctgcact tgcaatcccg ctggcagcct ggacacctgt gacccccgca gtgggcgctg
1441 cccctgcaaa gagaatgtgg aaggcaacct atgtgacaga tgtcgcccgg ggacctttaa
1501 cctgcagccc cacaatccag ctggctgcag cagctgtttc tgctatggcc actccaaggt
1561 gtgcgcgtcc actgcccagt tccaggtgca tcacatcctc agcgatttcc accagggagc
1621 cgaaggctgg tgggccagaa gtgtgggggg ctctgagcac tccccacaat ggagcccaaa
1681 tggggtcctc ctgagcccag aagacgagga ggagctcaca gcaccaggga agttcctggg
1741 agaccagcgg ttcagctatg ggcagccccc catactgacc ttccgggtgc ccccgggga
1801 ctccccactc cctgtacagc tgaggctgga agggacaggc ttggccctgt ccctgaggca
1861 ctctagcctg tctggccccc aggatgccag ggcatcccag ggaggtagag ctcaggttcc
1921 actgcaggag acctccgagg acgtggcccc tccactgccc cccttccact tccagcggct
1981 cctcgccaac ctgaccagcc tccgcctccg cgtcagtccc ggccccagcc ctgccggtcc
2041 agtgttcctg actgaggtcc ggctcacatc cgcccggcca gggctttccc cgccagcctc
2101 ctgggtggag atttgttcat gtcccactgg ctacacgggc cagttctgtg aatcctgtgc
2161 tccgggatac aagagggaga tgccacaggg gggtccctat gccagctgtg tccctgcac
2221 ctgtaaccag catggcacct gtgaccccaa cacagggatc tgtgtctgca gccaccatac
2281 cgagggccca tcctgtgaac gctgtttgcc aggtttctat ggcaaccctt tcgcgggcca
2341 agccgacgac tgccagccct gtccctgccc tggccagtcg gcctgtacga ccatcccaga
2401 gagcggggag gtggtgtgta cccactgccc cccgggccag agagggcggc gctgtgaggt
2461 ctgtgatgat ggctttttg gggacccgct ggggctcttt gggcaccccc agccctgcca
2521 ccagtgccag tgtagcggga acgtggaccc caatgccgtg ggcaactgtg acccctgtc
2581 tggccactgc ctgcgctgcc tgcacaacac cacgggtgac cactgtgagc actgtcagga
2641 aggcttctac gggagcgccc tggcccctcg acccgcagac aaatgcatgc cttgcagctg
2701 tcacccacag ggctcggtca gtgagcagat gccctgcgac ccagtgacag gccaatgctc
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2821 ccagcctggg aggggctgcc ggagctgcaa gtgtcaccca ctgggctccc aggaggacca
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2941 caggtgccag ctgggtttct tcggctcctc aatcaagggc tgccgggcct gcaggtgctc
3001 cccactgggc gctgcctcgg cccagtgcca ctataacggc acatgcgtgt gcaggcctgg
3061 cttcgagggc tacaaatgtg accgctgcca ctacaacttc ttcctcacgg cagacggcac
3121 acactgccag caatgtccgt cctgctacgc cctggtgaag gaggagacag ccaagctgaa
3181 ggccagactg actttgacgg aggggtggct ccaagggtcc gactgtggca gtccctgggg
3241 accactagac attctgctgg gagaggcccc aaggggggac gtctaccagg gccatcacct
3301 gcttccaggg gctcgggaag ccttcctgga gcagatgatg ggcctcgagg gtgctgtcaa
3361 ggccgcccgg gagcagctgc agaggctgaa caagggtgcc cgctgtgccc aggccggatc
3421 ccagaagacc tgcacccagc tggcagacct ggaggcagtg ctggagtcct cggaagagga
3481 gattctgcat gcagctgcca ttctcgcgtc tctggagatt cctcaggaag gtcccagtca

Figure 5B

INTERNATIONAL SEARCH REPORT

Inter l application No.
PCT/US01/18948

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12; 435/325, 471, 69.1, 252.3, 320.1, 71.1, 71.2; 530/412

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,610,031 A (BURGESON ET AL) 11 March 1997 (11/03/97), see entire document.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 2001	Date of mailing of the international search report 26 SEP 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

Intern[illegible] application No.
PCT/US01/13945

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 1/14, 14/47, 14/475; A61K 38/16, 38/17, 38/18; C12N 5/10, 15/12, 15/63, 15/64

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/2, 8, 12; 435/325, 471, 69.1, 252.3, 320.1, 71.1, 71.2; 530/412

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, CAS ONLINE, CAPLUS, MEDLINE
search terms: laminin-15, laminin alpha5, laminin beta2, laminin gamma3, recombinant production, isolation, administration, therapy, method, implant, photoreceptor, photosensitivity